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The workshop was the outgrowth of a collaborative venture of the Committee on Response Strategies to Unusual Chemical Hazards and a counterpart Italian Committee to inveszigate the effects of area-wide chemical contamination at Seveso, Italy. The workshop served as a conceptual framework for the development and refinement of investigative approaches to the study and prediction of the health impacts of multichemical exposures. The workshop attempted to elucidate the analytic, environmental, and toxicologic problems associated with chemical mixtures, to describe state-of-the-art investigational procedures, and to advance concepts and approaches for the understanding of multichemical interactions influencing chronic risks to human health. This volume contains the papers that served as a basis for discussions at that conference. The papers are grouped into three major categories: Identification of Analytic Issues, Environmental Interactions, and Toxicological Interactions of Mixtures in Humans and Laboratory Animals.

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Assessment of Multichemical Contamination

Proceedings of an International Workshop

Milan, Italy
April 28-30, 1981

B. Paccagnella, *Chairman*
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Committee on Response Strategies to Unusual Chemical Hazards
Board on Toxicology and Environmental Health Hazards
Assembly of Life Sciences
National Research Council

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This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

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PREFACE

In 1977, the National Academy of Sciences-National Research Council (NAS-NRC) was invited by the Italian government to join in a collaborative effort to investigate the effects of area-wide chemical contamination at Seveso, Italy. The contamination was the result of an explosion of a reaction vessel containing highly toxic 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD, commonly known as "dioxin"), which produced a cloud of chemical that was carried southward by the wind, exposing humans, animals, and plant life for several kilometers. The NAS-NRC sent a team of U.S. scientists to visit Italy and to determine with Italian officials the needs and opportunities for cooperative study. The NAS-NRC team recommended the development of a continuing relationship of U.S. and Italian scientists for the purposes of exchanging scientific and technical information, fostering the conduct of complementary research, organizing workshops and conferences to examine the impacts on health and the environment, and assisting in the coordination of exchange of scientists engaged in the analysis of this accident.

The NAS-NRC formally structured its involvement in this collaborative venture by establishing the Committee for Binational Cooperative Study of Exposure to TCDD, which was later renamed the Committee on Response Strategies to Unusual Chemical Hazards. The committee's terms of reference were twofold: first, the development of guidelines that might be used to implement a worldwide mechanism for guiding biomedical researchers at the scene of accidents similar to that at Seveso (thereby ensuring the most comprehensive collection of scientific information in a timely manner) and second, the evaluation--in cooperation with Italian counterpart scientists--of newer health data from the Seveso accident and the design of future studies.

One of the committee's first undertakings was a workshop, held in March 1980 at the National Academy of Sciences, on Plans for Clinical and Epidemiological Follow-Up After Area-Wide Chemical Contamination. The Proceedings from that workshop have been printed recently by the National Academy Press. During that workshop, the U.S. committee and Italian scientists met to discuss plans for a second workshop to be held in Italy in 1981. It was agreed that the second workshop would serve as a conceptual framework for the development and refinement of investigative approaches to the study and prediction of the health impacts of multichemical exposures. Thereupon, in April 1981, a contingent of international scientists met in Milan, Italy, to participate in the International Workshop on the Assessment of Multichemical Contamination. Specifically, it was the intent of the workshop to elucidate the analytic, environmental, and toxicologic problems associated with chemical mixtures, to describe state-of-the-art investigational procedures, and to advance concepts and approaches for the understanding of multichemical interactions influencing chronic risks to human health. This volume contains the papers that served as a basis for dicussions at that conference. The papers are grouped into three major categories: Identifcation of Analytic Issues, Environmental Interactions, and Toxicological Interactions of Mixtures in Humans and Laboratory Models.

PART I: IDENTIFICATION OF ANALYTIC ISSUES

Environmental Sampling Approaches and Procedures: A U.S. Perspective

Robert W. Risebrough, Brock W. de Lappe, and Wayman Walker II[1]

Within the United States, there is no consensus on the most appropriate procedures for the environmental monitoring of pollutant chemicals. This is equally true for programs that address problems associated with multichemical contamination and programs that address a single pollutant of particular concern. It is therefore difficult, if not impossible, to present in this paper a description of the U.S. perspective.

At the federal level, there are a number of monitoring programs for synthetic chemicals undertaken by several agencies, including the U.S. Fish and Wildlife Service, the Food and Drug Administration, the Environmental Protection Agency, and the National Oceanic and Atmospheric Administration. Frequently, there are parallel programs at the state level. Furthermore, perhaps more than in any other country, the private component has been actively involved in projects that assess the impact of pollutant chemicals upon human health, wildlife, and the integrity of the environment.

Although the rationale and the approaches may differ widely from program to program, there is a common element in that all

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have been developed in response to public concern over problems arising from the presence of synthetic chemicals in the environment. Almost always these are present as complex mixtures. Recent spectacular improvements in analytical methods have greatly increased the number of components within these mixtures that are detected. By providing us with a very different kind of data set, the new methods are forcing us to reexamine the concepts used over the past decade in our approach to environmental monitoring. Moreover, the accumulation of more than a decade's worth of data from past programs now permits an assessment of the extent to which these have fulfilled their mandates. The present symposium gives us, therefore, an opportunity to redefine some of the problems with a view toward developing new concepts and approaches.

Of necessity, we have prepared this paper on the basis of our own background and experience; we present, therefore, only one U.S. perspective on the problems of multichemical contamination. Our primarily ecological view acknowledges that man is the dominant member of the ecosystem. Moreover, it assumes a subtle, but important, difference between approaches that would protect human health and those that would protect other species and the integrity of the environment. In protecting wildlife we wish to maintain healthy populations. Mutations, birth defects, and cancers would not, except in extreme cases, affect the viability of a population. In protecting human health, however, we specifically wish to prevent such effects. Separate approaches to these problems are not mutually exclusive; instead, they are, to a very large extent, inclusive.

Theoretically, within an ecological perspective we attempt to look at the broad picture. In this context we suggest that some of the solutions implemented a decade ago, in response to the problems as they were then conceived, have now produced another kind of problem. For example, many thousands of measurements of trace elements in environmental samples are being undertaken in various pollutant-monitoring programs without determining or assessing the magnitude of the natural fluxes of these elements in the local environment. Frequently the anthropogenic component of such fluxes is minor, yet the trace elements are usually referred to as "pollutants," suggesting a more massive perturbation of environmental processes by man than is actually the case. Such measurements waste considerable sums of money without necessarily being responsive to the need to provide public protection.

Similarly, there are programs involving many thousands of measurements of synthetic organochlorine compounds, although the majority of these pollutants are now ubiquitously distributed so that their presence in the environment is no longer news. Invariably, only the pollutant compounds already known and readily identifiable are reported, even when the samples analyzed may contain many other components. But the existing data management systems almost always ignore the unidentified compounds. New approaches to environmental monitoring, therefore, should attempt to look at the complete picture, rather than only at those peaks on the chromatograms produced by known pollutants.

Our capability to detect an increasing number of synthetic organics in environmental samples has not been followed by an increase in our ability to assess their long-term significance. Unless there are biological effects, demonstrable now or in the future, monitoring programs will consist of little more than exercises in analytical chemistry and data reporting. Our inability to detect current biological effects is hardly proof, however, that they are not occurring. In developing new concepts in monitoring, we might consider methods for the detection of biological changes produced in response to a change in the chemical environment.

The past decade has witnessed a series of crises arising when a problem chemical has been detected in a local environment. How can we avert these crises, or at least anticipate them? Superimposed upon the other considerations, therefore, is the necessity to promote long-term protection by anticipating future problems, rather than merely reacting to the latest crisis. Using the past as a guide for the future, we shall present several case histories, reviewing how the chemicals currently recognized as problems were first detected in the environment.

THE DETECTION OF BIOLOGICAL CHANGE

Since so many variables affect the health of individual members of both human and wildlife populations, an effect produced by a new synthetic chemical in the environment, or by an interaction of several synthetic chemicals, might not be distinguished above the background for many years. Relatively little attention is now being paid to the problems of detecting such biological change in the environment at an early stage. Perhaps a future workshop will consider the problems of detection as well as the measurement of biological effects associated with changes in the chemical environment. Few such changes will be as dramatic as the rapid disappearance of many forms of aquatic life from freshwater environments of northeastern North America and southern Scandinavia, which are resulting from acid rain (Dochinger and Seliga, 1976; Hutchinson and Habas, 1980). These gross perturbations will undoubtedly be followed by more subtle effects, resulting from the rapid leaching of many minerals from the soil cover.

The thinning of eggshells of a number of bird species is a further example of a widespread biological change that has been exceptionally well-documented. The phenomenon appeared abruptly, on a global scale, in the years immediately following

1945, providing evidence of a significant alteration at that time. It was not detected, however, for 20 years (Hickey and Anderson, 1968; Ratcliffe, 1967). Such a totally unanticipated outcome would never have been included in any model designed to predict the environmental effects of a chemical. We wonder how many other still undetected effects have occurred.

Since some wildlife populations accumulate exceptionally high levels of nonpolar synthetic organic pollutants, abnormalities observed in these populations provide an indication of possible future hazard to humans. Seals and sea lions inhabiting coastal areas are among the species known to accumulate high levels of organochlorines. In recent years four very different kinds of reproductive abnormalities have been documented. On the California coast premature births have occurred in populations of the California sea lion (*Zalophus californianus*), Steller sea lions (*Eumetopias jubatus*), and harbor seals (*Phoca vitulina*)(DeLong *et al*., 1973; Gilmartin *et al*., 1976; Risebrough *et al*., 1980a). In Puget Sound, there have been birth defects in the local population of harbor seals (Arndt, 1973). In the Baltic, a low level of productivity in ringed seals (*Pusa hispida*) has resulted from a high incidence of pathological changes of the uterus among females of reproductive age (Helle *et al*., 1976a, 1976b). In the western portion of the Wadden Sea, pup production has been below normal, and initial juvenile mortality has increased in the local population of harbor seals (Reijnders, 1976, 1978). Cause-effect relationships remain obscure (Risebrough, 1979; Risebrough *et al.*, 1980a), yet these problems merit detailed investigations.

In the early 1970s, a number of birth defects occurred in colonies of fish-eating birds in Long Island Sound (Hays and Risebrough, 1972) and in Lake Ontario (Gilbertson *et al.*, 1976), suggesting the presence in local environments of chlorinated dioxins or similar teratogens (Bowes *et al.*, 1973). Recent improvements in analytical methods now permit the measurement in environmental samples of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. This compound has recently been detected in archived eggs of herring gulls (*Larus argentatus*) at levels sufficient to account for the observed abnormalities in Lake Ontario (D.J. Hallett, R.J. Norstrom, personal communication).

We should, therefore, continuously monitor wildlife populations to detect biological and associated chemical changes that might have implications for human health. But there is no standard method. Moreover, wildlife populations appear to be of little value in the detection of environmental carcinogens; high incidence of mortality resulting from such a cause would be extremely difficult to detect.

Environmental sampling is one approach toward finding the causes of the increased incidence of human cancers in certain areas that appear to be environmentally related. The development of sensitive biological tests for the presence of mutagens that are also potential carcinogens (Ames et al., 1975) permits the examination of extracts of air, water, or sentinel organisms from these areas. Since the amounts of material are not limiting, sample extracts that could provide a positive response to the test could be fractionated and studied in detail to determine which chemicals are the causative factors.

TOXIC CHEMICALS AND ENVIRONMENTAL CRISES: THE PAST DECADE

In revising our strategies for environmental sampling and monitoring, we hope primarily to avert or at least minimize the effect of future crises resulting from the unanticipated presence of toxic chemicals in the environment. We should, therefore, consider examples from the recent past in developing such strategies.

The Chlorinated Dibenzodioxins

The chlorinated dibenzodioxins, particularly 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), are of particular relevance in northern Italy, as a consequence of the explosion in Seveso that released significant quantities of TCDD into the local environment. TCDD is now recognized as an environmental problem chemical in Lake Ontario and in an area of Lake Huron, having entered the lakes as a component of industrial wastes and byproducts. Extensive contamination of the food webs of

the lakes has occurred over the past decade (D.J. Hallett and R.J. Norstrom, personal communication).

The first clues to the presence of this compound in Lake Ontario included a very high incidence of embryonic death in fish-eating birds in the late 1960s and early 1970s (Fox _et al_., 1975; Gilbertson, 1974; Gilbertson and Hale, 1974), as well as an apparently high incidence of birth defects (Gilbertson _et al_., 1976). These had prompted an earlier search for the presence of TCDD (Bowes _et al_., 1973), which has only more recently been detected through more sophisticated analytical techniques (D.J. Hallett and R.J. Norstrom, personal communication).

Because of its very low levels in environmental samples, TCDD has not been detected in routine analyses of mixtures of synthetic organic compounds. Rather, it has been detected only as a result of specific searches where its presence was suspected. These suspicions arose from a knowledge that processes involving 2,4,5-trichlorophenol are likely to produce this dioxin, and from the biological observations of a high incidence of embryonic death and an apparently high incidence of birth defects. Similar observations of biological abnormalities in the future might indicate the presence of other teratogens in the environment. Moreover, biological indicators would probably provide a more sensitive index than would chemical methods.

The DDT Compounds

A U.S. policy decision to prohibit any contamination of the milk supply by dichlorodiphenyltrichloroethane (DDT) and its derivatives probably comprised the factor that first led to the recognition that the DDT compounds had become significant environmental pollutants. In response to the need to enforce this tolerance level, and those imposed for other foods, analytical methods, particularly electron-capture gas chromatography, developed rapidly in the 1960s. The use of such methods in the monitoring of foods therefore provided the data that first indicated the widespread occurrence of p,p'-dichlorodiphenyl-2,2-dichloroethylene (DDE) in the environment.

The first of a series of biological changes that were later associated with DDE was the population reduction and local extirpation of the peregrine falcon (Falco peregrinus) in Britain, areas of western Europe, and eastern North America (Hickey, 1969; Ratcliffe, 1980). Initial observations of the local disappearance of the species were made in the mid-1960s. Comparable observations of a number of other species quickly followed, particularly with the discovery of eggshell thinning. Several research papers subsequently demonstrated that most if not all of the shell thinning, as well as the population declines, can be attributed to DDE (reviewed by U.S. Environmental Protection Agency, 1975).

Thus, a need to protect the food supply from "DDT" led to the discovery that a derivative compound, p,p'-DDE, was accumulating in local environments. Biological observations indicated the existence of widespread deleterious effects, which were only later shown to be caused by DDE.

The Polychlorinated Biphenyls

The development of electron-capture gas chromatography also led to the discovery that polychlorinated biphenyls (PCBs) were widespread environmental contaminants. These substances first appeared as unidentified peaks on gas chromatograms during analyses of environmental samples for DDT compounds. The unknown pollutants were detected at high levels in species showing patterns of local population decline, including the white-tailed eagle (*Haliaetus albicilla*) in Sweden and the peregrine falcon in western North America. Laboratory analyses, based on extensive programs of biological sampling undertaken in Sweden, the Netherlands, and North America, indicated that PCBs were just as widely distributed as DDT compounds (Jensen *et al.*, 1969; Koeman *et al.*, 1969; Risebrough *et al.*, 1968). The Yusho epidemic, which began in 1968, associated with PCBs as well as the contaminant chlorinated dibenzofurans (Kuratsune *et al.*, 1976), demonstrated the human health significance of these compounds and prompted measures to phase out their use. A systematic effort to identify principal unknown peaks on gas chromatograms, before it was known that they represented any particular hazard, resulted therefore in a significant environmental discovery.

Dieldrin and Related Pesticides

Several countries, including the United States, have discontinued or severely restricted the use of dieldrin and other pesticides, including heptachlor, the chlordane compounds, and endrin. Primarily, they are suspected of being potential human carcinogens. Their widespread occurrence in the environment, as shown in a number of monitoring programs, indicated that they had sufficient environmental stability to reach humans through the food webs. The history of this class of biocides provides the justification for a monitoring network that is repetitive over both time and space. This approach establishes a data bank that presents a picture of the geographical distribution of a particular biocidal compound in the environment, as well as changes in levels with time.

Mirex

Mirex, previously used in the southeastern United States to contol fire ants and now extensively used in tropical countries to control leaf-cutting ants, is a suspected human carcinogen. On this basis, this country has discontinued its use. Monitoring programs that provided data indicating both its mobility and stability in the environment played a key role in the decision.

Mirex is also among the many organochlorine contaminants of the Lake Ontario ecosystem. A program for multichemical pollutant analysis of environmental samples first identified it in fish (Kaiser, 1974). Subsequent studies revealed that mirex is a widespread contaminant throughout the Lake Ontario food webs (Hallett et al., 1976; Norstrom et al., 1980), originating from a factory discharge into the Niagara River. Mirex also turned up in marine mammal tissue in the Netherlands (Ten Noever de Brauw et al., 1973), where it had been used under another name as a fire retardant. Its detection was again the result of multichemical analyses of environmental samples.

Kepone

The contamination by kepone of the James River Estuary in the state of Virginia has led to severe restrictions on both commercial and recreational fishing in the area, causing extensive economic damage. This time, a monitoring program was not responsible for the detection of the presence of kepone in the local environment. Rather, workers at the manufacturing plant in Hopewell, Virginia, began to show symptoms of neurological illness, which were attributed in 1975 to kepone contamination. A preliminary survey revealed its widespread distribution in the local environment, as a result of factory discharge of kepone-contaminated waste into a local watershed (Huggett and Bender, 1980; Huggett et al., 1980).

Summary

There are a number of other examples, including the environmental contamination in Michigan by polybrominated biphenyls (PBBs) manufactured as fire retardants, but these case histories are sufficient to provide guidelines for revising our monitoring and sampling strategies:

1. systematic efforts to identify unknown peaks on gas chromatograms have played a major role in the detection of problem chemicals in the environment;
2. worthwhile results have been produced by programs that "go out and see" what is in the environment;
3. biological observations in many cases have provided the first indication of the presence of problem chemicals in the environment; and
4. important data on one contaminant have been obtained from programs designed to monitor other groups of compounds.

Identification of unknowns on gas chromatograms and determination of the environmental distribution of the corresponding pollutants should therefore be a part of future monitoring strategies. Furthermore, our approach should look at the broadest possible spectrum of the pollutant chemicals detected, whether or not all are currently identified.

THE DATA BASE

Data Collection

Over the past 10 years or more, U.S. monitoring programs have examined the distribution of synthetic organic compounds, principally pesticides, in various environmental components including soils (Carey, 1979; Carey et al., 1978, 1979); total diet samples, comprising the "market basket" survey (Johnson and Manske, 1976); the wing muscles of waterfowl (White, 1979b; White and Heath, 1976); freshwater fish (Veith *et al.*, 1979); starlings (*Sturnus vulgaris*)(White, 1979a); tissues of bald eagles (*Haliaetus leucocephalus*) (Kaiser *et al.*, 1980); and coastal and estuarine mollusks, principally *Mytilus* (Butler, 1973; Butler *et al.*, 1978; Goldberg *et al.*, 1978). The results of these programs and others of shorter duration have been the subject of many research papers and reports, so that a significant paper glut has developed. Attempts to computerize data within some programs have begun, but relatively little progress has been made in rapid retrieval of information from a data base common to more than one program.

So far these programs have reported only the identified compounds. The results on the printed page rarely provide an indication of the relative abundance of unidentified compounds.

Within our own perspective we shall comment principally on the Mussel Watch Program, which has continued the earlier efforts of Philip Butler (Butler, 1973; Butler *et al.*, 1978) to monitor a variety of pollutants in coastal waters, using mussels (*Mytilus* sp.) and other bivalves as indicator organisms. In 1976, the first year of this program, only the DDT and PCB compounds among the organochlorines were reported (Goldberg *et al.*, 1978). Studies the second year revealed chlordane and hexachlorocyclohexane compounds, hexachlorobenzene, dieldrin, and endrin in many of the samples. During the third year, analyses were conducted by capillary column electron capture, as well as by flame-ionization-detector gas chromatography. Adoption of this method has caused a crisis in data management, which will extend to the other monitoring programs as the capillary techniques are adopted as routine. Figures 1 and 2 illustrate the nature of this problem; they depict the aromatic fraction of extracts of mussels (*Mytilus edulis*) obtained from Boston Harbor and oysters (*Crassostrea virginica*) from Cape Charles, Virginia. The majority of the peaks represent unknown substances; the three other fractions obtained in the analytical separation also contain many unknown pollutants detected by capillary column electron-capture chromatography. Each analysis may therefore yield information on more than 100 individual compounds. In reporting data on only the few identified, we ignore significant quantities of information; the number of individual compounds detected, however, imposes major constraints in the storage and reporting of this information.

Data Management

A monitoring strategy for the 1980s cannot be implemented without consideration of a data management system. In part, the U.S. Mussel Watch Program is tackling the problem in the following way: Each of the resolved peaks is assigned a Kovats index for the column type employed. This index, developed in flame-ionization-detector gas chromatography, is based on the relative emergence time of the n-alkanes. Thus, n-C_{17} is

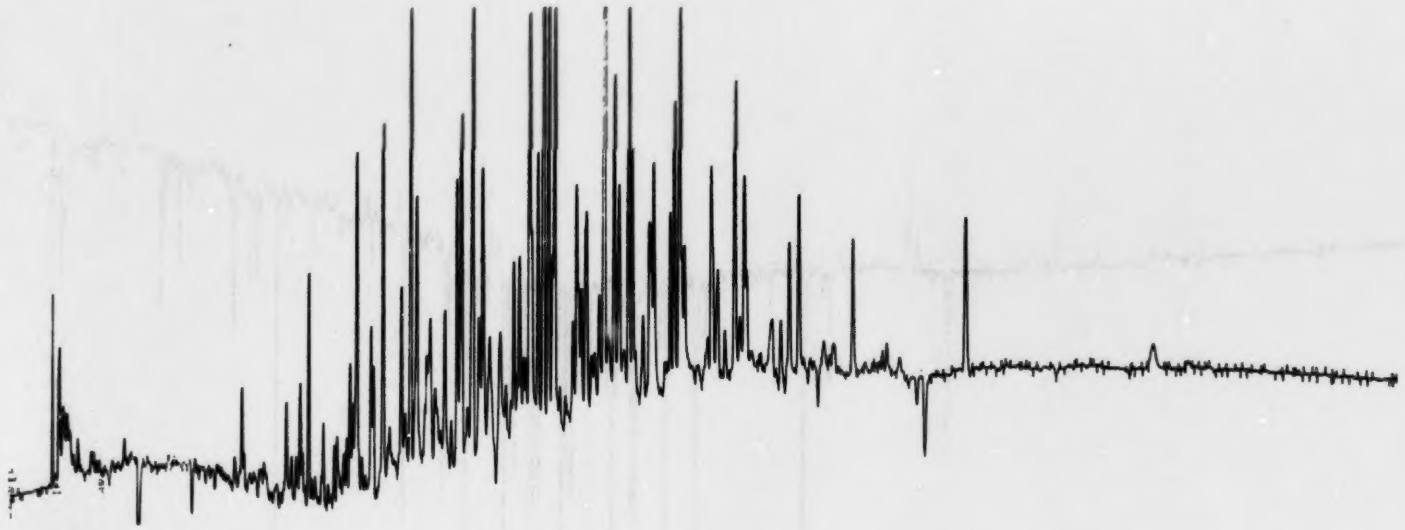

FIGURE 1. Electron-capture chromatogram of aromatic (F_2) fraction, extract of mussels from Boston Harbor, 25 September 1978, National Mussel Watch Program, Bodega Marine Laboratory. 30 m SE-54 fused silica column, Carlo Erba 2150 gas chromatograph. This fraction is one of four obtained in the separation procedure. More than half of the peaks represent currently unidentified compounds.

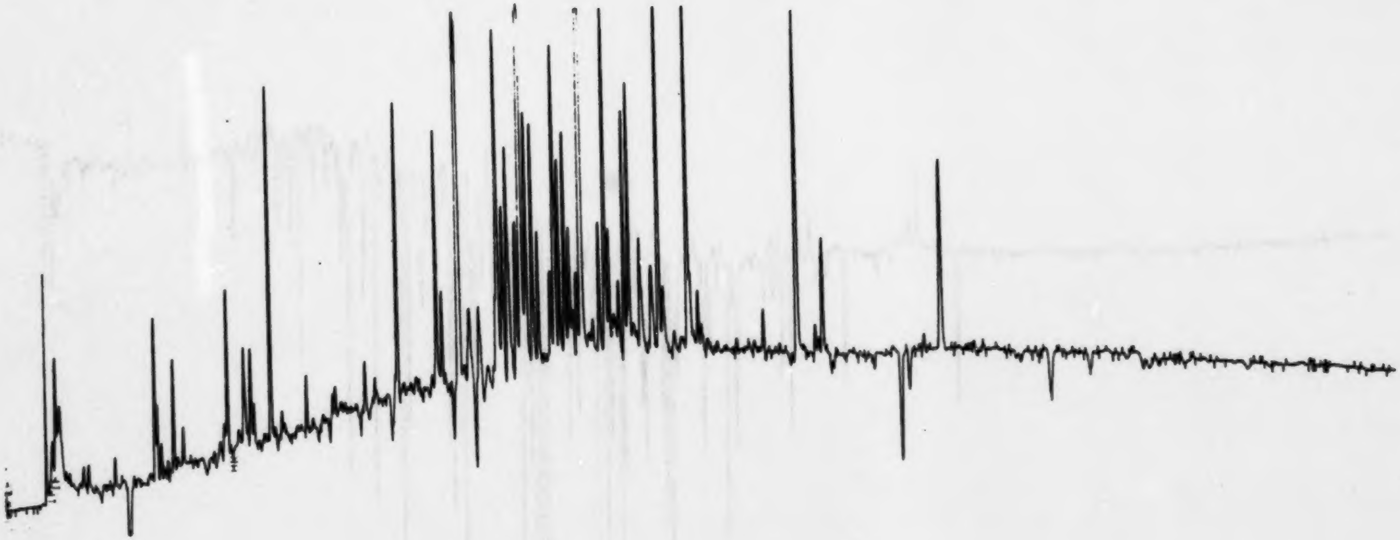

FIGURE 2. Aromatic (F_2) fraction, extract of oysters, *Crassostrea virginica*, from Cape Charles, Virginia, 21 October 1978, National Mussel Watch Program, Bodega Marine Laboratory. 30 m SE-54 fused silica column, Carlo Erba 2150 gas chromatograph. The majority of the peaks represent currently unidentified compounds.

assigned a Kovats index of 1700, n-C_{18} a Kovats index of 1800. On the SE-30 column, pristane emerges at one-tenth of the distance between the two alkanes and is thereby assigned a Kovats index of 1710 for this column type. Coinjection of alkanes and the various synthetic organics permits assignment of a Kovats index for the latter, which can then be used as a basis for the assignment of indices to unidentified peaks on the electron-capture chromatograms. A computer program permits the calculation of Kovats indices based on retention times and of an estimate of concentration from the integrated areas, frequently based on the response factor of an internal standard. Labels are assigned as compounds are identified by gas chromatography/mass spectrometry or other techniques.

Further developments in this area will no doubt include storing all relevant sample information, including collection data, the derived data produced by the current generation of gas chromatographic outputs, as well as the gas chromatographic signal. Additional expansion of the data base further indicates that the concept of producing tabulations of all data on paper is becoming obsolete. Computerization of the data base permits retrieval of only those data needed for a specific purpose, making hardcopy outputs only as required. Thus, the data management approaches of the 1970s will not apply to the 1980s.

MONITORING STRATEGIES: SOME GENERAL CONSIDERATIONS

Monitoring programs designed to address specific questions, and to obtain a data set relevant to those questions, are more likely to be successful than those that gather data in a "look and see" approach to environmental surveillance (National Academy of Sciences, 1980). The U.S. national monitoring programs generally address the distribution of persistent pesticide residues in environmental components, including soil, river waters, freshwater fish, terrestrial birds, the wing muscles of waterfowl, and the tissues of the bald eagle, an endangered species that is at the top of a food web that includes fish. To date, the Mussel Watch Program has operated more in a research mode than in a monitoring mode, primarily consisting of a "look and see" operation. The data obtained on pollutant levels in bivalves in the coastal zone, however, should help us to implement long-term policies for protection and management.

The Mussel Watch Program shares with other national programs a strategy that dictates that measurements be repeated over both space and time. Spatial measurements not only assist in pinpointing input sources of pollutants, but they also assist in the interpretation of the significance of local pollution levels. Thus, the existence of a data set from San Francisco Bay, which can be compared to data from other areas of the California coast as well as the rest of the country and the world, is useful in examining the hypothesis that one or a combination of pollutants might be responsible for lesions observed in a local species of fish. If other bays and estuaries have comparable levels of particular pollutants and the local fish exhibit no such lesions, this would suggest that these pollutants do not play a major role in causing the lesions.

Measurements over time permit determinations of increases or decreases in environmental levels in response to increased input or to administrative or other actions that decrease environmental input. Thus, the Mussel Watch Program has determined that levels of DDE and PCBs in the California coastal environment have declined by up to an order of magnitude over the past decade (Risebrough _et al_., 1980b).

The expansion of the data set, and the impossibility of managing these data on paper, emphasize the necessity for a greater coordination among the existing programs. If a new problem chemical is identified in the environment, it would be advantageous to search an extensive data file to determine if it might be one of the previously observed "unknowns."

In this brief paper, we have not mentioned sampling strategy and the problems of reducing sampling variance and of ensuring that samples taken are a good representation of the environmental component being monitored. The approaches taken and progress made in the Mussel Watch Program have been presented in the _Proceedings_ of the Barcelona Mussel Watch Workshop (National Academy of Sciences, 1980) and in a summary of the Mussel Watch Program in California (Risebrough _et_ _al_., 1980b).

The high economic cost incurred through the loss of food resources and employment as a result of toxic chemical contamination over the past decade would alone justify continuing surveillance programs. Furthermore, there are the unmeasureable risks to human health and the potential damage to wildlife populations, which cannot be quantified. It would appear, however, that a greater level of coordination among the participating institutions is required to achieve a successful solution to the problems we now face.

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Comprehensiveness and Separation of Samples

Milos Novotny[1]

A multitude of toxic substances produced during various technological processes dictates a continuous development of effective analytical methods for assessing potential health hazards. In such a way, modern analytical chemistry becomes involved in the processes of recognition, evaluation, and control of chemical hazards.

The analytical problems associated with chemical contamination of the environment vary from one case to another. Specific procedures can determine known toxic substances even in extremely complex sample matrices. Frequently, the necessary methodology will have impressive sensitivities and precision, often down to part-per-trillion levels. Very small amounts of selected pollutants must often be measured with high sensitivity and accuracy to assess the potential for personal exposure, bioaccumulation, metabolism, or the compound's persistence in the environment. Knowing what substances to look for simplifies the process, and some knowledge of the related industrial process can also be helpful in elucidation of any additional toxic substances, such as starting materials and by-products.

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The release of toxic dioxins into the environment on various occasions and the subsequent human exposure (see Holmstedt, 1980, for a review) are examples of this type of situation. A chemist's solution will almost always involve highly selective instrumental techniques that "ignore" the remaining sample constituents. Though expensive, such techniques can provide adequate quantitative answers without extensive sample fractionation. The well-established mass-fragmentographic approach or the recently advocated mass spectrometry/mass spectrometry methods (Kondrat and Cooks, 1978) have this general capability or potential.

Multichemical contamination situations are significantly different. If the area of toxic hazard is not sufficiently defined, additional strains are placed on both sampling and analytical measurement procedures to ascertain comprehensiveness. Even the best available sample separation schemes and measurement methods are challenged when one endeavors to determine what environmentally important substances are contained in a sample of either a suspected health hazard or a proven toxicity.

Complications are numerous, as illustrated by the Love Canal story (Axelrod, 1980), among others, in which numerous industrial by-products in different quantities were deposited at one place over a period of time. In such instances, the original complexity can be magnified by various additional reactions among the mixture components, which, in turn, may multiply the number of compounds to be included in analytical as well as toxicological considerations (e.g., various synergistic effects). This chain reaction is likely to continue due to weathering, oxidations, and interactions with the ecological system. Naturally, some detoxification processes may take place, but the opposite can also be true, forming more and/or other highly toxic substances.

Such a scenario is a nightmare for toxicologists, epidemiologists, and analytical chemists alike. One cannot accurately predict either the occurrence or the concentrations of the toxic substances. Even sample components present in lesser (or even trace) amounts cannot necessarily be labeled as toxicologically unimportant.

Therefore, although extensive toxicological screening and specialized tests will provide the most important information on the hazards of a given multichemical contamination source, one must simultaneously conduct a detailed chemical characterization, given the limitations of methodology and cost. This is a formidable task: the sample components may span a respectable range of molecular weights, polarities, and other chemical features. Certain biological effects could be derived from organic pollutants, toxic metals, or a combination of both. Different toxic substances can be present with a large dynamic range of concentration. If sufficiently simple, specific analytical screening methods for the compounds of known toxicity can be undertaken for orientation purposes.

However, these procedures cannot substitute for a more comprehensive approach to sample characterization, which should include (a) a comprehensive extraction; (b) fractionation into different compound classes; (c) chromatographic separation and detection of the individual sample components; and (d) positive identification and quantitation of the environmentally important sample components within a specific concentration range. Such general analytical methods have a precedent in modern analysis of biological materials, in which samples of comparable molecular complexity are frequently encountered. Certain directions in environmental trace analysis, initiated several years ago, have similar methodological goals. The U.S. Environmental Protection Agency has been working on the so-called Master Analytical Scheme (Donaldson and Garrison, 1979; U.S. Environmental Protection Agency, 1981). Another approach, for sample screening for more than 100 pollutants in common water samples (U.S. Environmental Protection Agency, 1979), attempts to develop a general protocol for analytical survey and regulatory purposes. Although the Master Analytical Scheme has been criticized (Ehmann et al., 1979; Golton, 1979) for its potential problems, the reasons for a standard, unified approach are fully justified. In this paper I will evaluate, in general terms, some procedures that could lead to a comprehensive characterization of environmental mixtures pertaining to multichemical contamination. I will also discuss state-of-the-art analytical methods.

SAMPLE PREPARATION AND FRACTIONATION

Any successful screening for environmentally important compounds, in either qualitative or quantitative terms, must begin with adequate sample preparation. The analysis could cover a variety of materials, including contaminated soil or water, waste oils, plant materials, and animal tissue, so an adequate sample homogenization and exhaustive extraction must be ascertained first. Here, there are only a few general guidelines, since there are usually some variations from one sample to another.

Sample preparation strategy depends also on the roughly expected amounts of toxic substances. Extraction is a relatively straightforward task, if one deals with solid wastes or material directly from a chemical dump. Even in the vicinity of a major contamination site, the concentrations of pollutants will be at least in the part-per-million range. At much lower levels, from a few parts-per-million to parts-per-trillion (limits given by the current sensitivity of modern instrumentation), legitimate concerns arise about the efficiency of extraction and sample losses due to the clean-up procedures.

Solvent partition is generally recommended for recovery and concentration of organic pollutants from aqueous media, whereas the Soxhlet extraction is suitable for various pulverized materials. Solvents of a great eluting strength cover a wide range of organic compounds to be extracted. A few definite studies concern extraction efficiency of different solvents, but methylene chloride seems to be generally favored as a solvent of adequate purity, low boiling point, and good extraction capability. Other solvents are occasionally used for specific purposes.

Alternative methods should exist for polar organic compounds, which are not readily extractable from aqueous media with organic solvents. During the development of new methods, the efficiency of extraction and other preliminary sample treatments are frequently checked with isotopically labeled compounds carried through the entire sample-preparation procedures. Obviously, the proper choice of internal standards may be crucial to overall accuracy. Ideally, there should be multiple standards for determining several different compounds. At best, this is an impractical proposition, and some compromises must be met in practice.

When toxic pollutants of interest are encountered in aqueous media, there are additional means of sample concentration. One can trap trace organics on various solid granular materials while pumping an appropriate amount of a contaminated sample through them. Thus, the substances of interest are collected on "accumulator columns" packed with activated carbon, inorganic adsorbents, polyurethane foams, porous organic polymers, and reversed-phase packings (see reviews in Garrison et al., 1979; Keith, 1976). Under suitable conditions, such materials strongly retain organic trace components, which can later be recovered for further separation, characterization, or quantitative analysis. There are similar procedures for trapping relatively volatile compounds purged with a gas.

After accumulating sufficient sample amounts, one can analyze the content of the sampling tubes. Recovery procedures may involve either thermal desorption into a gas-chromatographic column, in case of volatile substances, or solvent extraction. Quantitative evaluation of the content of accumulator columns requires careful standardization (Novak *et al*., 1965; 1979).

In most cases of multichemical contamination, the mixtures of extracted organic compounds will be complex. Compound identification and/or reliable quantitation require chromatographic analyses at high resolution. However, even the best chromatographic systems will not provide complete separation of all possible mixture components and reliably determine the compounds of interest in complex sample matrices. Thus, there must be further fractionation of these extracts or otherwise preconcentrated mixtures.

Various classes of compounds can be obtained through an appropriate analytical fractionation scheme. Even selective enrichment is sometimes feasible if the compounds contain some unique structural features. This type of sample fractionation is sometimes synonymous with "sample clean-up," as in the removal of ballast materials during low-effiency chromatography or solvent partition steps in any trace analysis of agriculturally important chemicals. Further examples of more or less selective fractionations involve the use of ion-exchange materials to retain acidic or basic substances, or "extraction" of volatile chemicals from nonvolatile matrices with a stream of gas and adsorption on porous polymers.

Relatively effective fractionation procedures are based on solvent partition schemes, which separate the mixtures according to acid-base properties and polarities (see Figure 1 for an example). This general approach was originally developed for fractionation of tobacco smoke, one of the most incredibly complex mixtures, but it can be adapted to other analytical problems. Each of the separated fractions is then subjected to detailed chromatographic investigation. Depending on relative representations of the components, which vary from one case to another, certain overlap among the fractions may occur, and further "clean-up" may be desirable. Alternative analytical schemes may be based on various forms of column chromatography (gels, ion-exchangers, or adsorbents), but some concerns exist about possible losses of various compounds on the columns.

The compounds of interest may cover a wide range of volatility and polarity. Consequently, highly efficient forms of both gas and liquid chromatography should be complementary rather than competitive approaches to a satisfactory analysis,

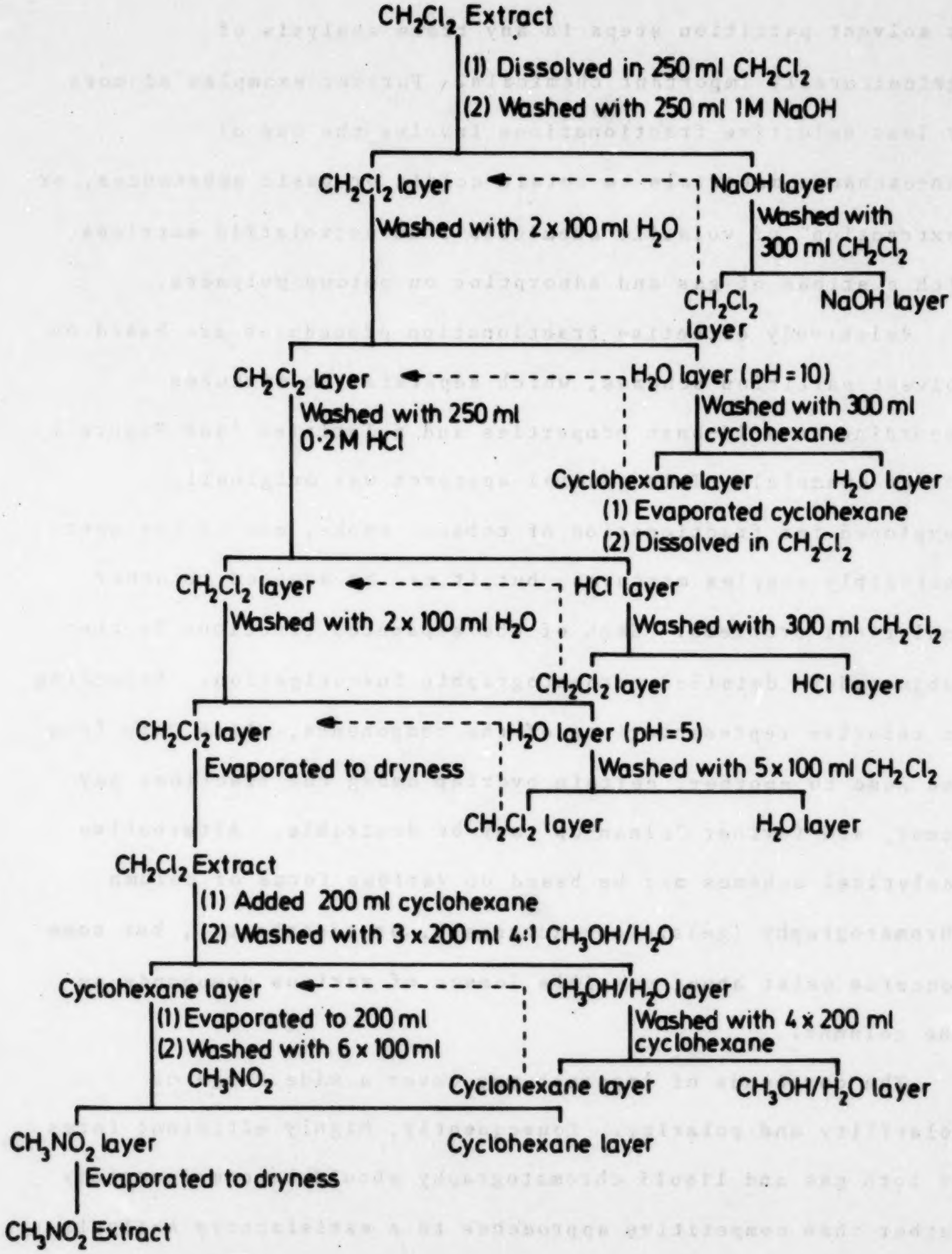
CH$_2$Cl$_2$ Extract
(1) Dissolved in 250 ml CH$_2$Cl$_2$
(2) Washed with 250 ml 1M NaOH
CH$_2$Cl$_2$ layer
NaOH layer
Washed with 2 x 100 ml H$_2$O
Washed with 300 ml CH$_2$Cl$_2$
CH$_2$Cl$_2$ layer
NaOH layer
CH$_2$Cl$_2$ layer
H$_2$O layer (pH = 10)
Washed with 250 ml 0·2 M HCl
Washed with 300 ml cyclohexane
Cyclohexane layer
H$_2$O layer
(1) Evaporated cyclohexane
(2) Dissolved in CH$_2$Cl$_2$
CH$_2$Cl$_2$ layer
HCl layer
Washed with 2 x 100 ml H$_2$O
Washed with 300 ml CH$_2$Cl$_2$
CH$_2$Cl$_2$ layer
HCl layer
CH$_2$Cl$_2$ layer
H$_2$O layer (pH = 5)
Evaporated to dryness
Washed with 5 x 100 ml CH$_2$Cl$_2$
CH$_2$Cl$_2$ layer
H$_2$O layer
CH$_2$Cl$_2$ Extract
(1) Added 200 ml cyclohexane
(2) Washed with 3 x 200 ml 4:1 CH$_3$OH/H$_2$O
Cyclohexane layer
CH$_3$OH/H$_2$O layer
(1) Evaporated to 200 ml
(2) Washed with 6 x 100 ml CH$_3$NO$_2$
Washed with 4 x 200 ml cyclohexane
Cyclohexane layer
CH$_3$OH/H$_2$O layer
CH$_3$NO$_2$ layer
Cyclohexane layer
Evaporated to dryness
CH$_3$NO$_2$ Extract

FIGURE 1. Solvent partition scheme for complex mixtures. (From Novotny et al., 1981. Reproduced with permission of IPC Science and Technology Press, Ltd.)

especially because only some 10 to 20% of the components in environmental extracts are sufficiently volatile and amenable to gas-chromatographic analysis.

For the sake of completeness, samples of a suspected or proven environmental hazard should also be screened for the presence or absence of certain toxic elements, such as heavy metals. Here, the necessary method for enrichment and analytical measurements is vastly different from the other approaches. The modern methods of simultaneous multi-element analysis are fully capable of determinations within a wide range of concentrations. Among them, inductively coupled plasma spectroscopy appears most prominent (for a review, see Dahlquist and Knoll, 1978). Direct sample analyses are often feasible; however, sample preconcentration is sometimes required for analysis (see reviews in Gould, 1968; Minczewski, 1967). When toxic elements are found at environmentally alarming levels through a multi-element atomic spectral technique, further studies are in order to elucidate the speciation aspects.

GAS-CHROMATOGRAPHIC ANALYSIS

Sample Volatility

Modern gas chromatography spans an impressive range of sample volatility. The advent of thin-film capillary columns and thermally stable stationary phases has enabled analysis of relatively large molecules in the gas phase, provided that they are stable enough themselves at column temperatures extending up to 350°C. Under such circumstances, molecules as large as alkanes with carbon number over 50, certain triglycerides, and eight-ring polyaromatic compounds, can be successfully chromatographed. Figure 2 shows an example of a mixture of polycyclic aromatic molecules chromatographed on a thermally stable capillary column (Hirata *et al*., 1981).

Modern gas chromatography, therefore, can analyze numerous toxicologically important compounds, including many industrial and agricultural chemicals, chlorinated pesticides, dioxins, and various other, possibly carcinogenic compounds. The more polar types of these substances may, however, suffer from decomposition problems during gas chromatography due to their limited stability and undesirable interactions with the analytical systems. These problems are particularly evident with very low sample quantities. Then high-performance liquid chromatography (HPLC) would be a better analytical alternative, but the problems of universal detection and sensitivity still exist.

The volatility range of gas chromatography can be extended by chemical derivatization techniques in certain cases. Thus, many polar and nonvolatile compounds can be quantitatively transformed into derivatives amenable to gas chromatography, an approach well-established in biomedical analyses (see the relatively comprehensive reviews of different derivatization techniques in Drozd, 1975; Knapp, 1979). There are also a limited number of environmental applications of this general

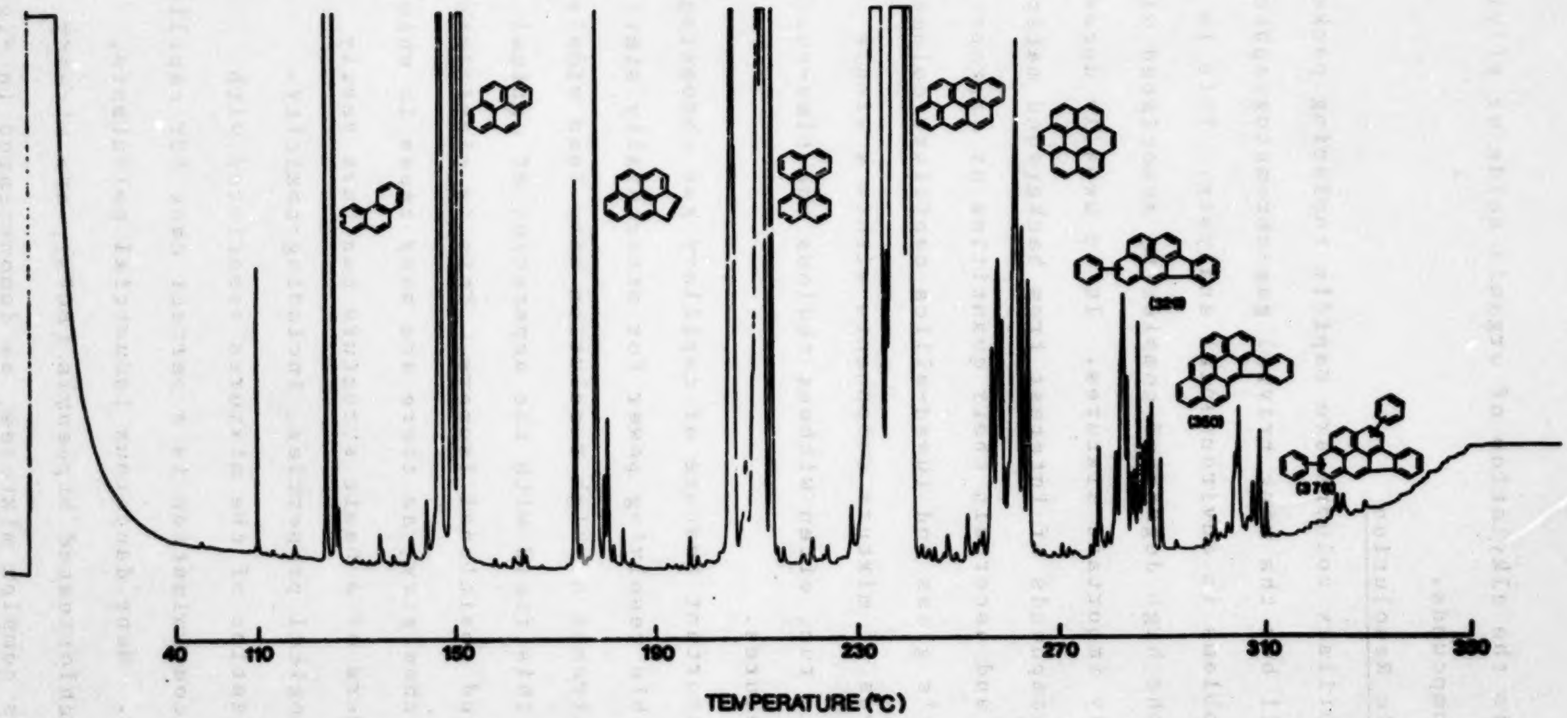

FIGURE 2. High-temperature trace analysis of polynuclear aromatic compounds extracted from carbon black. (From Hirata et al., 1981. Reproduced with permission of Elsevier Publishing Company)

approach, as in the alkylation of organic acids or silylation of phenolic compounds.

Chromatographic Resolution

Today, capillary columns are rapidly replacing packed columns for all but the most trivial gas-chromatographic separation problems in environmental analysis. This is due primarily to the high degree of complexity associated with environmentally important mixtures. It is usually necessary to separate the compounds of interest from background materials to identify them and ascertain their quantities at appropriate levels. Today's glass and fused-silica capillary columns can resolve hundreds of mixture components within a single chromatographic run, often without tedious and time-consuming clean-up procedures.

Another important feature of capillary gas chromatography is its remarkable resolving power for structurally similar compounds. Extremes of high resolution have been widely documented in this field with the separation of optical isomers or *cis*-*trans* and positional isomers. This is significant for environmental chemistry, as there are many cases in which different isomers of a basic structure can have vastly different biological properties, including toxicity.

Characterization of the mixtures associated with multichemical contamination is a perfect case for capillary gas chromatography. Many dangerous industrial pollutants, including polychlorinated biphenyls (PCBs), are already manufactured as complex mixtures, as demonstrated in Figure 3

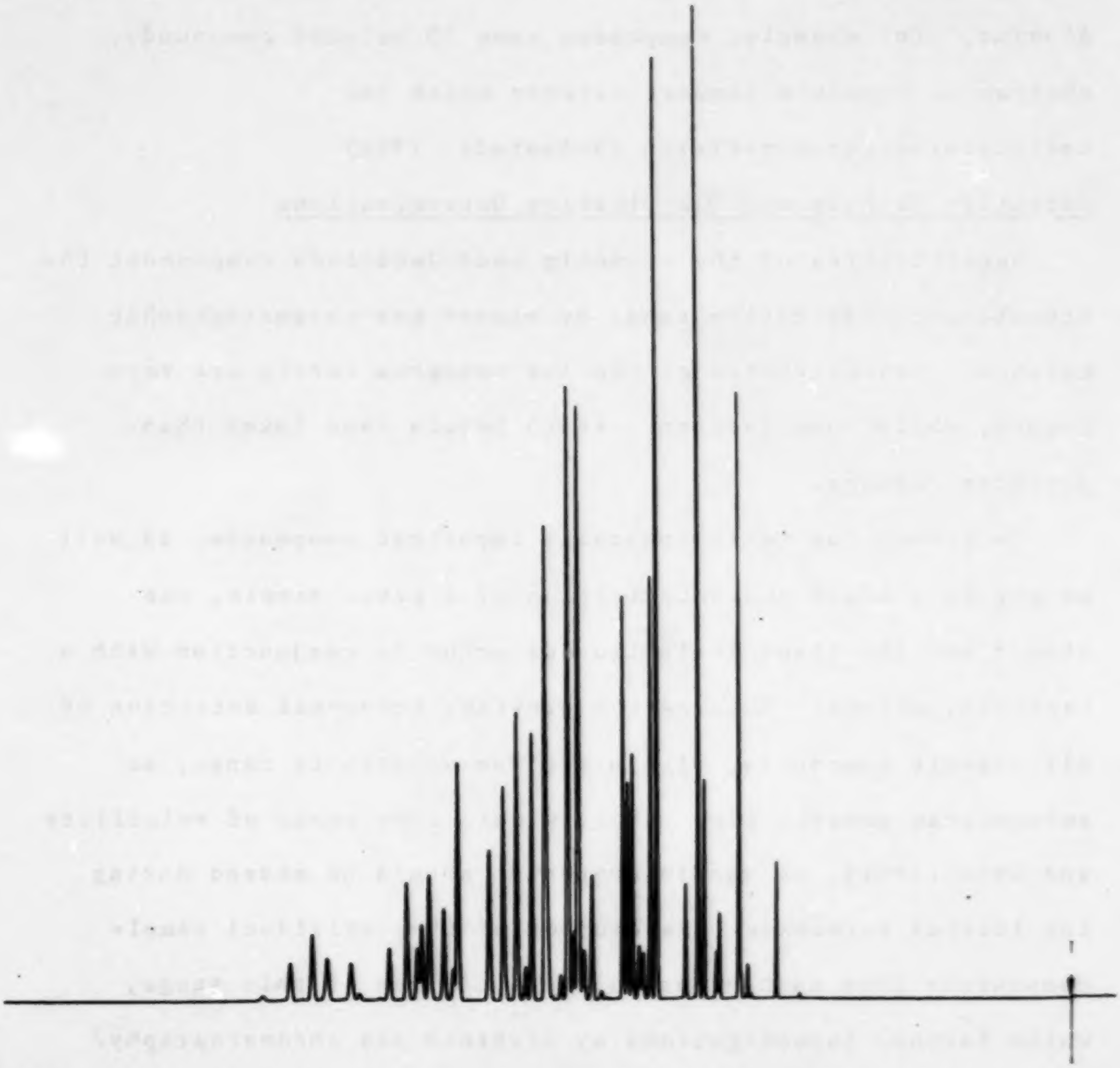

FIGURE 3. Gas chromatographic separation of isomeric polychlorinated biphenyls (Aroclor 1016) at 220° C. (From Schomburg et al., 1974. Reproduced with permission of Elsevier Publishing Company)

(Schomburg et al., 1974). Incidental blends of dangerous chemicals created at chemical dump sites also require the highest available component resolution. The term "chlorinated dioxins," for example, comprises some 75 related compounds, whereas 22 possible isomers already exist for tetrachlorodibenzo-p-dioxin (Holmstedt, 1980).

Detection Methods and Quantitative Determinations

Sensitivities of the commonly used detectors complement the operating concentration range of modern gas-chromatographic columns. Sensitivities at the low nanogram levels are very common, while some detectors reach levels even lower than picogram amounts.

To screen for toxicologically important compounds, as well as giving a broad characterization of a given sample, one should use the flame ionization detector in conjunction with a capillary column. This device provides universal detection of all organic compounds, within a given volatility range, at subnanogram sensitivity. Thus, within that range of volatility and sensitivity, no sample component should be missed during the initial screening. Resolution of the individual sample components from each other can be optimized at this stage, while further investigations by combined gas chromatography/ mass spectrometry are in order.

In the past, the major incentive for developing gas-chromatographic selective detectors has involved overcoming the problems of sample complexity with packed columns. These detectors are "blind" to compounds in a mixture that do not possess certain unique structural features, i.e., chromophores or heteroatoms. But these detectors and capillary columns significantly enhance each others' capabilities (see Table 1 for the sensitivities and some other features of the most common selective detectors).

Since parallel use of the flame ionization detector and certain selective detectors is now widespread, perhaps similar equipment should be used in sample screening and characterization for multichemical contamination cases. Many environmentally important compounds have structural features that are compatible with these detectors. Figure 4 (Grob, 1975) shows an example in which the flame ionization and electron capture detectors are used as a means of complementary detection: the electron capture recording suggests the presence of trace amounts of PCBs, while the flame ionization detector indicates a complex mixture (as might be expected with this sample type).

Various techniques in gas chromatography/mass spectrometry provide the utmost in detection selectivity. Their utilization may range from the common spectral scanning mode for compound identification to measurements of preselected ions arising from suspected pollutants. The commonly used techniques of mass chromatography provide valuable information on the presence and the levels of different compound classes. This paper will not discuss these methods in detail, but will emphasize two major

TABLE 1

Properties of Some Gas Chromatography Selective Detectors

Detector	Selectivity mode	Approximate sensitivity (g)
Electron capture	Affinity to low-energy electrons	$10^{-13} - 10^{-14}$
Thermionic	Nitrogen	10^{-12}
	Phosphorus	10^{-13}
Flame photometric	Sulfur	10^{-9}
	Phosphorus	10^{-11}
Electrolytic conductivity	Halogen compounds	10^{-10}
Ultraviolet	Aromatics	10^{-9}
Photoionization	Partially enhanced response to certain organic molecules as compared to flame ionization (not truly selective)	$10^{-11} - 10^{-12}$

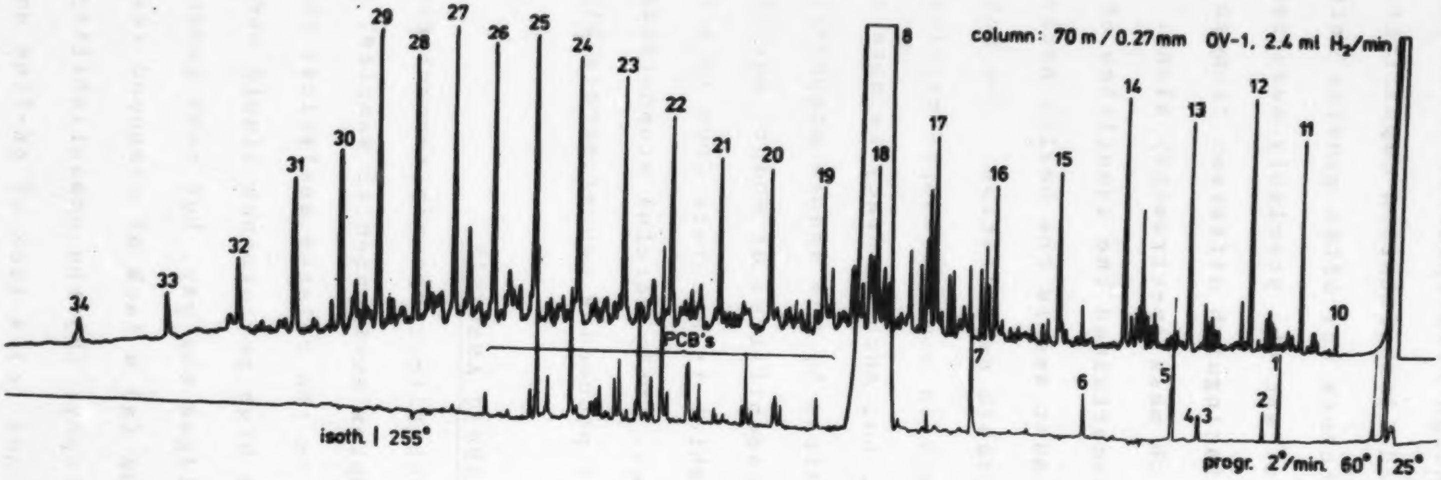

FIGURE 4. Chromatographic analysis of the nonpolar fraction of sewage extract. The organic components are simultaneously detected with the flame ionization detector (upper chart) and the electron capture detector (lower chart). PCB's = polychlorinated biphenyls. (From Grob, 1975. Reproduced with permission of Pergamon Press)

points: (a) even though mass-spectral techniques can give the ultimate in compound identification capabilities, screening with selective detectors may often provide initial information and supporting data; and (b) precisely measured chromatographic data help one to distinguish different isomers, which is difficult to do with mass spectrometry alone.

Once one has ascertained the identities of sample constituents, one must assess the health hazard for a given sample through reliable quantitation. The subject of measurement accuracy in complex sample matrices goes far beyond this presentation, but such accuracy is more often significantly impaired by the sample preparation steps than by the quantitative capabilities of modern gas-chromatographic instrumentation, which is accurate down to a few percent with calibrated mixtures. Only careful standardization of the overall analytical procedure can ascertain highly quantitative results.

LIQUID CHROMATOGRAPHIC ANALYSIS

Although nonvolatile compounds account for a large percentage of complex environmental samples, many major weaknesses exist in the related analytical chemistry. The rapid advances in high performance liquid chromatography (HPLC) have somewhat bridged the gap, but many general problems still persist, including (a) a lack of compound resolution comparable to gas chromatography; (b) the unavailability of universal detection means; and (c) a lack of on-line ancillary techniques for compound identification. The frontier areas of modern analytical chemistry will be associated with development in these directions for some time.

HPLC techniques are highly valuable for certain environmental problems. Many industrial toxic substances are relatively nonvolatile (see, e.g., Hites and Lopez-Avila, 1979). Although acquiring HPLC separations at optimum levels is less straightforward due to mobile-phase variations, the method development for a limited number of known environmental pollutants can be a relatively easy task. On the other hand, screening efforts in this direction are likely to be more of a problem.

Resolving capabilities of HPLC have been recently improved through developments in microcolumn technology (for reviews, see Novotny, 1980; Scott, 1980). Efficiencies comparable to those routinely achieved in capillary gas chromatography are now available, but the analysis times in micro-HPLC are considerably longer. To overcome this problem, a significant reduction in the particle size for packed columns or the column radius for capillary HPLC columns must become feasible; both alternatives present a formidable technological challenge.

Furthermore, there is no universal sensitive detector for HPLC. In a number of practical analyses, this difficulty is overcome by the availability of certain selective detectors. Detection devices based on absorption spectrophotometry, spectrofluorimetry, and electrochemical phenomena are popular.

Some of these devices have detection sensitivities down to 10^{-12} grams, but such figures vary widely for different compounds. In a few cases, one can enhance detection sensitivity through a chemical alteration of solutes, and pre- and postcolumn derivatizations are becoming increasingly common.

However, identification problems with nonvolatile solutes separated by HPLC are particularly worrisome. Clearly, HPLC cannot match the availability of ancillary techniques for gas chromatography. Although possible utilization of liquid chromatography/mass spectrometry was vaguely mentioned during discussion of the Analytical Master Scheme (Donaldson and Garrison, 1979), it is not likely that such a combination will be fully developed for some time (Arpino and Guiochon, 1979). Development of various spectroscopic and electrochemical techniques for compound identification in HPLC is one of the most important tasks of modern analytical chemistry.

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The Characterization of Complex Mixtures Including Natural and Xenobiotic Organic Substances

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Many of the problems still faced in the daily pursuit of research aimed at elucidation of the molecular nature and function or dysfunction of living systems and their interactions with our biosphere concern the analysis of selected components in complex mixtures of organic substances or the identification of all of the components of the complex mixtures themselves. More often than not the components of interest are present only in trace levels, placing particular constraints on suitable methodology.

All biological systems utilize, modify, and excrete complex mixtures, which comprise a natural milieu. Some components originating from these natural sources can produce toxic effects in other species within the biological community (Kingsbury, 1980; Oehme et al., 1980). Superimposed on such a natural molecular environment (Eglinton et al., 1979) is an evergrowing assortment of anthropogenically derived complex mixtures (Horman, 1979; Risebrough, 1982; Safe, 1979). Some components are of concern because of their threat to the general ecological well being of our biosphere (Bowes, 1981); others may have the potential of continuing risk to human health (Burlingame et al., 1980; Horman, 1981; R.W. Miller, 1981;

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S.A. Miller, 1981; Richmond, 1981). In some cases, such components are known; in others, they are not (Risebrough, 1982).

Many general problems are encountered in studies of environmental chemistry of complex mixtures: they involve sampling and the matrix, contamination control, interlaboratory comparisons of complex mixture analyses, and correlations of known or unknown components from sample to sample and from environment to environment. In the context of potential risk to human health, there is then the determination of the extent of human exposure and its correlation with observed symptomatology. So, is the hard work really concentrated on devising the strategy for isolation, separation, and identification of complex mixtures? Is it in making an unambiguous link between one specific substance and the observed toxicity or between some small groups of substances and the observed toxicity? Or is the hard work ahead really empirical determination of the structure-toxicity relationships mediated by each species' metabolic individuality (the analog of structure-activity relationships in a pharmacological context)?

These are but a few of the difficult questions that only time and further knowledge can bring into better focus. At present, we know that particular physicochemical instrumental techniques have been the most successful and show the most promise among our analytical tools for providing clear answers, at least in (1) the chemical identification of the components of such complex mixtures, and (2) the metabolic activation of xenobiotic covalent lesion in cellular constituents. Since we are generally concerned with limited sample size and high analytical sensitivity, as well as concomitant molecular structure identification specificity, mass spectrometry in some form or other has become preeminent in this role in concert with gas and liquid chromatography (Burlingame et al., 1980). This established position will continue and even grow in scope in the foreseeable future due to rapid technical advances in mass spectrometry.

However, a couple of important aspects concerned with identifying complex mixtures and elucidating the molecular mechanisms of toxicity and carcinogenesis pose problems for mass spectrometry alone and require the use of additional and supporting techniques such as chromatography and Fourier transform nuclear magnetic resonance spectroscopy. One of these aspects is the ability to distinguish isomers or congeners, such as for polychlorodioxins and the polyhalogenated biphenyls where the toxicity and potency of the mixture depend in dramatic ways on each congener's halogen substitution pattern on the aromatic rings as well as their relative quantities (Aust et al., in press; Goldstein, 1979). The substitution patterns are best determined by nuclear magnetic resonance or highly reproducible chromatographic retention indices obtained from standards, where sufficient chromatographic resolution permits the necessary high precision retention index measurement. The determination of precise stereochemistry is also a matter for nuclear magnetic resonance, helped sometimes by structural analogs and retention index information, as, for example, with the ultimate carcinogen associated with the hydrocarbon, benzo(a)pyrene (Yang et al., 1976).

Another significant aspect is the potentially toxic substance or mixture, which varies from species to species. Often this process produces an extensive mixture of metabolites (Gelboin et al., 1977), some of which are relatively stable electrophiles with the nasty habit of covalently binding to cellular nucleophiles (Miller and Miller, 1977). The elucidation of their detailed chemical reactivity and mechanisms provides insight to permit understanding of the molecular basis of the nature of the toxicity observed, expressed primarily as covalent binding to a variety of cellular constituents, including proteins and the genetic encyclopedia, deoxyribonucleic acid (DNA). Therefore, from the points of view of both environmental exposure and intermediary metabolism, we face complex mixtures, some components of which covalently interact with cell machinery and express the toxicity syndrome with which we are eventually concerned.

Mass spectrometry has had a long and successful history in the study of complex mixtures. To my knowledge, the first work on mass spectrometric mixture analysis was done in 1940 by Hoover and Washburn (1940, 1941; Washburn et al., 1943). Using 1 hour of instrument time, they were able to analyze a nine-component mixture of C-5 and C-6 hydrocarbons in just 4 hours. The then-existing standard methods of separation using a fractionating column and a refractive index detector method would have taken 210 hours for the same mixture. The subsequent long, extensive record of the development of mass spectrometry for petroleum refining mixture analysis (Hamming and Foster, 1972) culminates in the utilization of low voltage electron impact techniques with high mass resolution to deal with the considerable heteroatom content of the various components of different crude oils (Lumpkin and Aczel, 1978). The invention of gas chromatography by James and Martin (1951, 1952) was essentially coincident with the recognition by Stevenson and Wagner (1951), Ryhage and Stenhagen (1963), McLafferty (1957), and Biemann (1962) (among others) that a mass spectrum consisted of an ensemble of gas-phase carbonium ion/radical chemical species characteristic of the original structure of the neutral molecule introduced into the mass spectrometer. Development of this inherent potential for mass spectrometry as a structural tool for very small sample sizes thereupon exploded into the tremendously large analytical and research effort that exists today (Burlingame _et al_., 1980, in press; Mellon, 1981; Waller and Dermer, 1980).

The first coupling of gas-liquid chromatography to mass spectrometry was attempted in 1957 (Holmes and Morrell, 1957). The coupling of scientific computers to help chemists deal with the tremendous load of data on the nature of complex mixtures began to be used in the mid-1960s (Chapman, 1978), and other more chemically mild ("soft") methods of creating gas-phase molecular ions began and still continue to develop at a vigorous pace (Morris, 1981). These include the use of ion-molecule reactions ("chemical ionization"), of high field gradients to effect electron tunneling ("field ionization"), thermalized electron plasma ("negative chemical ionization" or electron attachment to electronegative species), and primary photon (laser) atom or ion bombardment. Also playing important roles are accurate mass measurement, for direct determination of the elemental composition of molecules and mixtures, and higher mass resolution and their usage in complex mixture analysis (Kimble, 1978; Meili _et al_., 1979). In the mid-1970s, the development of high pressure liquid chromatography was initiated and various attempts were made to couple it with mass spectrometry (Mellon, 1981). The coupled techniques are not yet analytically mature.

For all the known and unknown natural and anthropogenic substances that we would want to identify and study including xenobiotics covalently bound to cellular constituents, there are three basic categories: mixtures that are volatile or volatizable through chemical derivatization procedures;

mixtures of thermally and/or chemically labile substances and salts, which we may not yet have learned how to derivatize without sample loss or decomposition; and substances that can be isolated but require degradative procedures in order to generate mixtures of substances that fall into the first two categories. These degradative procedures can, of course, be mild and highly selective, thereby avoiding the creation of artifacts. Examples are the very careful enzymic procedures for carcinogens or drugs covalently bound to native DNA (Straub and Burlingame, 1981). They can also be very crude and disrupt the structural integrity of the molecules being investigated, e.g., the pyrolysis of whole cells, kerogen, and plastics (Jones and Cramer, 1977).

Techniques of fused silica capillary gas chromatography coupled with both nominal (Burlingame et al., 1980, 1982; Mellon, 1981) and high resolution mass spectrometry (Meili et al., 1979) for complete identification of complex mixtures in the first category have been developed to a high level of routine performance. Present advances in field desorption and fast neutral atom or ion bombardment techniques with double focusing mass spectrometers have made studies of free substances in the second category eminently feasible and tractable while concurrently extending the molecular size into the 3,000 to 5,000 dalton range (Burlingame et al., 1982). The third category must still be addressed experimentally by some degradative approach amenable to separation and identification by techniques used in the first and second categories. Clearly the eventual understanding of the molecular nature of toxicity in its most general sense will require dealing with samples and mixtures in all three contexts.

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Standardization, Validation, and Quality Control

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The introduction of sophisticated chemical instruments into the analytical laboratory in recent years has considerably enhanced our knowledge of the distribution of chemical pollutants in the environment. Techniques such as gas chromatography/mass spectrometry now enable us to identify extremely toxic compounds at picograms per kilogram (parts per trillion) levels. However, for these measurements to have any validity, analysts must adopt rigorous protocols to establish the qualitative and quantitative limits of the analytical method being used.

From the qualitative viewpoint, there are a number of examples in the literature where investigators have misidentified chemical pollutants. In 1957, severe mortality occurred among chickens from certain areas in the South and Midwest of the United States. Scientists eventually traced the source of the disease to a fat supplement in the chickens' diets. An intensive research program over several years resulted in the isolation of the toxic factor as a crystalline material. Using a combination of ultraviolet and infrared spectroscopy together with low-resolution mass spectrometry, the investigators initially identified the crystalline material as a partially saturated chlorinated phenanthrene of molecular formula, $C_{14}H_{10}Cl_6$ (Wooten and Courchene, 1964). In fact, x-ray spectroscopy later identified the compound as 1,2,3,7,8,9-hexachlorodibenzo-p-dioxin (Contrell et al., 1966). Proposal of the incorrect structure was a result of a failure to recognize the aromatic ether absorption bond in the infrared spectrum. This conclusion that oxygen was not present in the molecule led to additional misinterpretation of the mass spectral data. Scientists attributed fragement ions resulting from the loss of 63 and 126 mass units to the loss of C_2H_4Cl and $2(-C_2H_4Cl)$, respectively. In fact these fragment ions are due to the loss of $-COCl$ and $2(-COCl)$, respectively. In retrospect, the analysts could probably have deduced the correct molecular formula by using high-resolution mass spectrometry, a technique which was then in its infancy but which has now proved to be one of the most powerful tools for the analysis of complex chemical mixtures.

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In routine analyses for known compounds, problems of accurate quantitation are often more severe than those concerning qualitative identification. This is particularly true in trace organic analysis, where investigators use multistep cleanup procedures to isolate the compound (or compounds) in question. In one interlaboratory study, 10 to 21 laboratories analyzed oyster tissue homogenates for three organochlorine pesticides [σ-benzene hexachloride (σ-BHC), γ-benzene hexachloride (γ-BHC), and dieldrin] (Hertz *et al.*, 1978). Relative standard deviations varied from 200% for σ-BHC to 87% for dieldrin. Obviously data of this nature are not useful. This paper, outlines what I consider some of the major factors that could lead to an improvement in environmental analyses.

THE ANALYTICAL PROCEDURE

Sample

The analyst very often has no direct involvement in the sampling program. This is unfortunate since the collection, storage, and handling of samples are obviously starting points for improvement in the reliability of environmental analyses. In cases that involve multichemical analyses, these considerations may be of even greater importance because of variations in physical and chemical properties. I will not discuss here appropriate sampling methods in detail because they are the subject of another paper in this conference.

Analytical Personnel

Although it seems self-evident, the qualifications of the analyst are of paramount importance in determining the success or failure of an analytical procedure. This is true even for so-called routine analyses. If they are conducted in a "cookbook" fashion, the analyst may fail to pay attention to important details, such as ensuring that the sample is not lost in solvent evaporation steps. Although the analyst may not have a complete understanding of statistics, he or she should have an elementary knowledge sufficient to carry on an intelligent dialogue with a trained statistician.

Methods

Selection of the appropriate method depends on both the nature of the sample matrix and the purpose for which the analytical result is intended. In terms of matrix, a method of analysis for an organic pollutant in biological tissues may be quite inappropriate for separating and analyzing the same pollutant if it is present on flyash. Concerning the end use of the data, if the purpose is to screen a large number of samples where there is no background information on the pollutant(s), a simple, rapid procedure such as radioimmunoassay may suffice. On the other hand, when a detailed knowledge of pollutant levels is required for health assessment decisions, the analyst may need to use a relatively sophisticated method that is not subject to interference from other compounds.

Quantitation

The instrument response (S) is related to the chemical compound concentration (C) by the equation, $S = g(C)$, where the response factor (g) is determined by instrumental calibration with standard compounds. Traditionally, the chemical compound's concentration is corrected for recovery losses that occur during sample preparation. Under these circumstances reliable results can be obtained only when the recovery is high (>50%) and consistent. However, with the widespread use of ion-monitoring mass spectrometry, investigators can add compounds labeled with stable isotopes prior to cleanup. Both the unlabeled compound and its isotopically labeled analog will then incur similar losses through the procedure, so that the analyst can determine the unlabeled compound concentration by a simple ratio calculation:

$$C = x \div y\ (RC_i),$$

where C is the unlabeled compound concentration, C_i is the concentration of isotope-labeled internal standard, x is the peak height for unlabeled compound ion, y is the peak height for internal standard, and R is the relative response of internal standard and unlabeled compound. In cases where the isotope-labeled internal standard gives an instrumental response for the unlabeled compound and vice versa, the equation is modified to read:

$$C = [(x - R_a y) \div (y - R_b x)]\ RC_i,$$

where R_a is the relative abundance of unlabeled ion to labeled ion in the internal standard, and R_b is the relative abundance of labeled ion to unlabeled ion in the unlabeled compound.

The isotope-ratio method can in fact be used to calculate the chemical compound levels reliably when recoveries are extremely low, as evidenced by recent data obtained in our laboratory. We fortified fireplace soot at two different levels with 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and 2,3,7,8-tetrachlorodibenzofuran (TCDF), together with [U-^{13}C]-TCDD[1] as an internal standard (Smith *et al*., 1981). We then determined recoveries after isomer-specific cleanup procedures relative to external standards of 2,3,7,8-TCDD and 2,3,7,8-TCDF, and we calculated the absolute level of 2,3,7,8-TCDD from the internal standard ratio method (see Table 1). Although the recovery levels, based on external standards, were extremely low due to the complex cleanup procedure, the ratio results for 2,3,7,8-TCDD were within 10% of the fortification levels.

We conducted similar analyses on replicate samples of carbonaceous material formed during the course of a transformer explosion (see Table 2). There was only a 4% variaton between the levels of 2,3,7,8-TCDD calculated for each replicate; however, calculations for 2,3,7,8-TCDF, which were based on the use of external standards and a 13% recovery factor, showed considerable variation.

APPLICATION OF QUALITY CONTROL TECHNIQUES

The quantitative error in any analytical determination is a composite of the precision or random error and the systematic

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[1] TCDD uniformly labeled with ^{13}C atoms.

TABLE 1

Recovery of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,7,8-Tetrachlorodibenzofuran (TCDF) from Fireplace Soot[a]

Sample No.	Fortification Level (µg/kg) TCDD	Fortification Level (µg/kg) TCDF	Recovery % TCDD	Recovery % TCDF	TCDD Ratio Calculation (µg/kg)
1	8	15	5.6	18	9.0
2	0.08	0.15	4.0	5.5	0.09

[a] Data from Smith *et al.*, 1981.

TABLE 2

Levels of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) and 2,3,7,8-Tetrachlorodibenzofuran (TCDF) in Binghamton Transformer Soot[a]

	Analytical Result (μg/kg)		
Sample	TCDD	TCDF	^{13}C-TCDD Recovery (%)
Environmental blank[b]	ND(<0.012)	ND(<0.085)	14
Binghamton soot #1	2.80	273	27
Fireplace soot	0.003	0.007	c
Binghamton soot #2	2.90	124	16

[a] Data from Smith *et al.*, 1981.

[b] Based on a theoretical sample weight of 3.5 mg.

[c] Not calculated because there was an error in sample trapping during the high-performance liquid chromatography step.

or bias error. The random error results from day-to-day variations in uncontrollable variables, such as laboratory temperature, and is inherent in any measurement process. Since this error can be equally positive or negative, analysts can adequately describe it by statistical procedures applicable to a Gaussian distribution.

The systematic error is of considerably greater importance to the chemist, as it introduces an error which is fixed in direction. The objectives of a quality control program are to define the precision and bias of an analytical method and to ensure that the estalished error limits are not exceeded during the analysis of environmental samples (American Chemical Society, 1980; Youden and Steiner, 1975).

Standards

The availability of analytical standards is a prerequisite to developing valid methods of analysis. Investigators must establish the purity of the compounds before using them. The nature of the data required for this purpose will vary with the properties of the compound. For organic compounds a full scan mass, ultraviolet or infrared spectrum should be a minimum requirement. Although this information is generally available either from the supplier or from experiments conducted in the analytical laboratory, publications or reports describing analytical methods frequently omit it.

In addition to using isotope-labeled compounds for internal standards, investigators often add them in substantial quantities to serve as carriers for the sample through the cleanup process. Under these circumstances, the isotopic purity of the internal standard becomes a limiting factor in trace-level analysis. If the labeled atoms are not of high enough isotopic purity, significant amounts of unlabeled compound may contaminate the standard.

Standard reference materials, containing defined levels of chemical compounds in a natural matrix, can also play a very important role in the development of reliable analytical methods. The National Bureau of Standards (NBS) has been involved for a number of years in providing these materials for inorganic compounds. A recent review article by scientists from NBS (Hertz *et al.*, 1978) pointed out that the greatest difficulty in extending them to organic compounds involves the lack of suitable methods for certification. However, in view of the present emphasis on trace organic analysis, standard reference materials for organic compounds should become available in the future.

Instrument Calibration

Calibration consists of measuring signals from the analysis of defined quantities of a standard compound. To determine the error associated with this measurement process, investigators should measure at least three different concentrations of the sample in triplicate with the concentrations covering the entire range of those expected. If the response is linear, the analysts can plot the data as a regression line. When we use ion-monitoring mass spectrometry to analyze TCDD in our laboratory, we have invariably found that we can plot results from the analysis of standard solutions over a dynamic range of 10^3 as linear calibration curves that pass through or close to the origin. Under these circumstances, a single-point ratio calculation is sufficient information for determining a response factor. However, if the calibration curve has a significant intercept, bias errors will be present, unless we make the calculations of sample concentrations directly from the calibration curve (Cardone _et_ _al_., 1980).

Although it is proabably unnecessary to prepare a separate calibration curve on a daily basis, we should analyze at least one standard every day. We can then plot the result as a quality control chart; any signficant deviation in the instrument response may then necessitate a reevaluation of the entire calibration curve.

Sample Categories

Scientists generally evaluate the reliability of the complete analytical procedure (sample collection, storage, extraction, cleanup, and instrument response) using the following sample categories: laboratory or procedural blanks, field blanks, and fortified field blanks. Three or more replications are necessary for the blank samples and for several of the fortification levels in order to obtain data on the precision of the method. Although we can address the effects of instrumental analysis separately by calibration with standards, Albro (1979) has suggested that analytical chemists often do not adequately consider extraction and cleanup as distinct steps.

A frequently erroneous assumption when samples are "spiked" (fortified) is that the spiking compound equilibrates with or distributes in the sample matrix in the same manner as the endogenous compound. If this does not occur, analysts may report artificially high recoveries. In the case of biological samples, radiolabeled compounds can often be administered *in vivo*, provided either that the compound does not metabolize or, in the event metabolism does occur, that the metabolites are not present in significant quantities in the tissue. We can then determine extraction efficiency by comparing the radioactivity after complete tissue destruction with the radioactivity obtained from the extraction procedure under evaluation.

With regard to validation of the cleanup method, if possible, we should evaluate each step in the method separately for recovery, reproducibility, and removal of interferences. We can then consider omitting those steps where recovery losses are not accompanied by a significant reduction in interfering compounds or sample matrix. During the course of analysis of field samples, we should intersperse validation type samples among them as quality control checks. There is a wide spectrum of opinion as to what constitutes the appropriate number of quality control samples, but 10% of the number of field samples is probably a reasonable number.

Limit of Detection

The concept of a detection limit (K) is based on the need to determine the minimum instrumental response from a sample that can be reliably detected. A descriptive equation relates the gross sample signal ($S\tau$), the field blank signal (S_b), and the variability in the field blank (σ_b): $S - S_b \geq K\sigma_b$. For convenience in measuring the limit of detection of individual samples, σ_b is generally replaced by the peak-to-peak noise in the area of the signal ($\sigma = \sigma\eta$). Various values of K have been proposed (Currie, 1968; Kaiser, 1970), but a committee of the American Chemical Society (1980) has suggested a value of K = 3, which seems acceptable since the error associated with making a false positive or false negative decision is only 7%. However, it is difficult to obtain quantitative accuracy near the limit of detection. To obtain more accurate results, an additional limit, the limit of quantitation, should be established with a value of K = 10.

Acceptable Total Error

The total error is a combination of the bias error and the precision errors. To make an objective evaluation of different analytical methods, McFarren _et al_. (1970) have recommended that investigators judge the total error at the 95% confidence level by using the following formula: total error = $100\ (d + 2S) \div \mu$, where d is the absolute value of the mean error, S is the standard deviation, and μ is the correct value. They considered methods that had total errors of 25% or less as "excellent," and methods with total errors between 25% and 50% as "acceptable." Eckschlager (1972) has pointed out that, when d is not statistically different from zero, the equation reduces to: total error = $100(2S) \div \mu$. Midgley (1977) has also suggested that 2S may include more than 95% of the results and that analysts should use either 1.7S or 1.8S, depending on the relative values of d and S. With these modifications, the total error formula could serve as a useful means of evaluating analytical methods.

Qualitative Confirmation

Investigators must adequately establish the identity of the sample based on the physical or chemical properties of the compound. For instance, in the case of halogenated compounds analyzed by mass spectroscopy or gas chromatography/mass spectroscopy, we can use isotope-ratio measurements to determine the number of halogen atoms. Additional analysis can improve confidence in any identification by using a method that differs in some significant aspect from the method used for quantitative measurement.

Interlaboratory Studies

After development and validation of a method within a given laboratory, general acceptance of the method depends upon comparison with other methods in an interlaboratory study. Two such studies of analytical methods for TCDD residues in biological tissues were conducted recently under the direction of U.S. federal agencies. In the first study, the

Environmental Protection Agency provided four laboratories with cleaned up extracts of beef fat, which had been fortified prior to cleanup with TCDD at levels ranging from 0 to 81 pg/kg together with a fixed quantity of [2,3,7,8-^{37}Cl]-TCDD[2] internal standard. All four laboratories conducted their analyses with mass spectrometers tuned to 10,000 resolution. Three used packed-column gas chromatography/mass spectrometry systems; the fourth introduced samples into the mass spectrometer by direct probe. Accuracy and precision were measured by regression analysis.

Only one laboratory was able to analyze TCDD with a high degree of precision and accuracy (see Figure 1) down to a fortification level of 9 pg/kg; the others obtained results lacking in both precision and accuracy, as illustrated in Figure 2. The only significant difference between the gas chromatography/mass spectrometry systems used by these laboratories was the fact that one (laboratory F) had a mass spectrometer with an ultimate resolution of 20,000, whereas the other (laboratory H) had one that could be tuned to a resolution of 150,000. Under these circumstances laboratory H could achieve a resolution of 10,000 with relatively wide slit widths and thereby carry out analyses at high sensitivity.

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[2] TCDD labeled with ^{37}Cl atoms at the 2,3,7, and 8 positions.

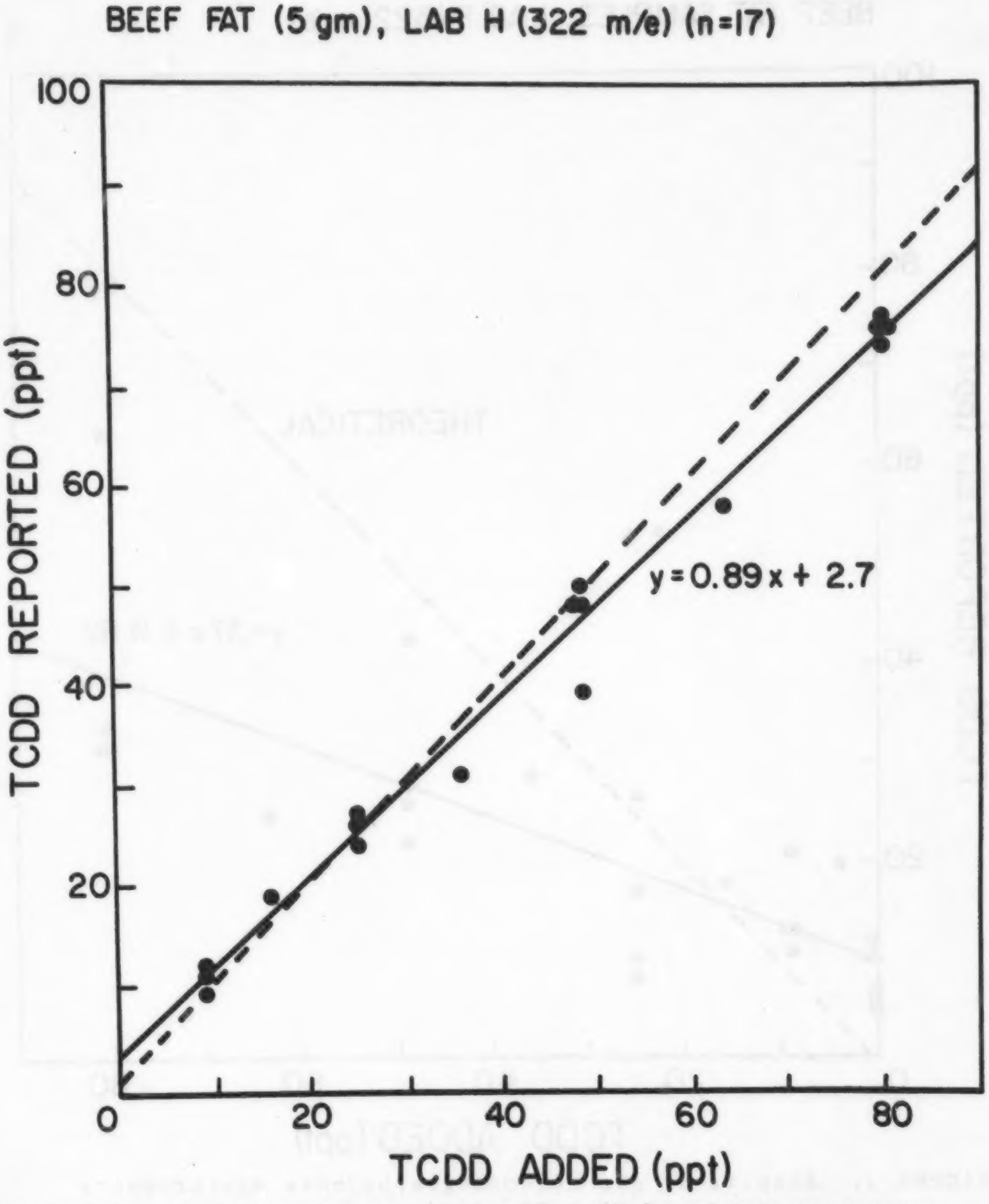

FIGURE 1. Results of gas chromatography/mass spectrometry analysis by "Lab H" of cleaned up extracts of beef fat, which had been fortified prior to cleanup with TCDD, plotted against theoretical result (broken line). (From Heath, 1979)

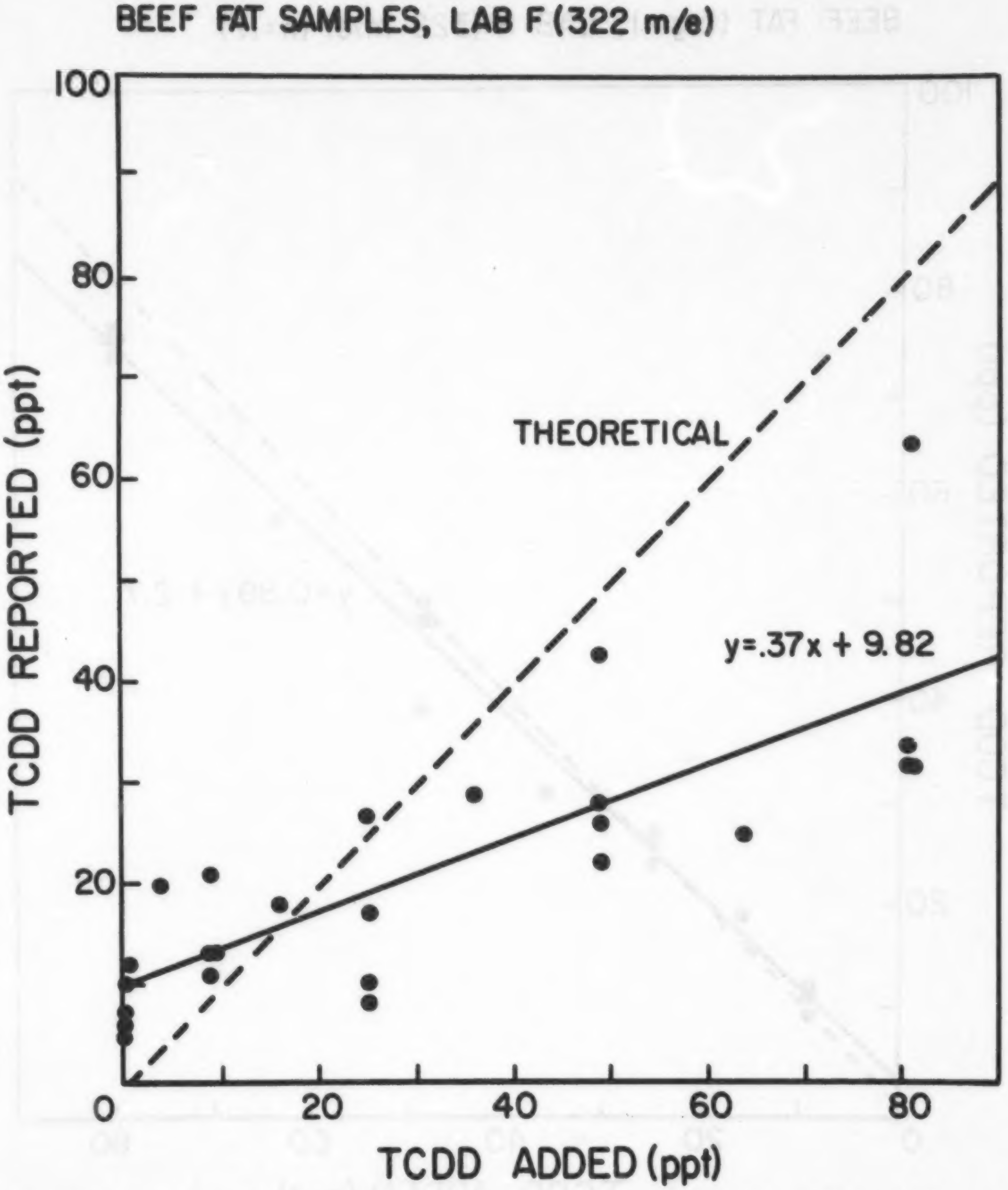

FIGURE 2. Results of gas chromatography/mass spectrometry analysis by "Lab F" of cleaned up extracts of beef fat, which had been fortified prior to cleanup with TCDD, plotted against theoretical result. (From Heath, 1979)

The second study focused on comparing the efficiency of various cleanup procedures, rather than evaluating the quantitative accuracy of instrumental methods of analysis (Brumby et al., 1981). Six laboratories received homogenates from fish collected in an area of potential TCDD contamination. Portions of the homogenates were fortified with TCDD, and the sample set consisted of the following:

Sample Number	Description of sample
1	sucker
2	sucker fortified with 105 (121) pg/kg TCDD
3	catfish
4	catfish fortified with 105 (121) pg/kg TCDD
5	catfish
6	catfish fortified with 105 (121) pg/kg TCDD

The cleaned up extracts were returned to the organizing laboratory for analysis by capillary gas chromatography/low resolution mass spectrometry. Twelve ions were monitored for confirmation of the presence of TCDD, and one of these ions, m/e 322, was used for quantitative measurements relative to the m/e 334 ion of the internal standard $[^{U-13}C]$-TCDD. Additional information on cleanup efficiency was provided by gas chromatography with an election-capture detector and full-scan gas chromatography/mass spectrometry.

Table 3 presents the ion-monitoring results. Three of the laboratories (C, F, G) provided cleaned up extracts in which TCDD could be quantitated and confirmed. Extracts from two

TABLE 3

Summary of Multiple-Ion-Detection Gas Chromatography/Mass Spectrometry Results of Study of Tetrachlorodibenzo-p-dioxin (TCDD) Extraction Cleanups at Seven Laboratories (A-G). (Confirmation of Identity; Quantitation in pg/kg)[a,b]

	A		B		C		D		E		F[f]		G	
Sample Number[c]	Conf.	Quant	Conf.	Quant.	Conf.	Quant.	Conf.	Quant.	Conf.	Quant.	Conf.	Quant.	Conf.	Quant.
1	No	5	No	6	No	-	No	-	No	-	N	-	No	9
2	No	67	No	89	Yes	77	No	-	No	-	Yes	67	Yes	47
3	No	34	No	42	Yes	57	No	-	No	-	Yes	25	Yes	22
4	No	188	No	99	Yes	128	d	d	No	-	Yes	113	Yes	117
5	e	e	No	53	Yes	38	d	d	d	d	Yes	45	Yes	56
6	No	178	No	199	Yes	107	d	d	d	d	Yes	100	Yes	96

[a] From Brumley *et al*., 1981.

[b] Confirmation of identity of TCDD occurs if the responses of the 12 monitored ions for the sample extract are consistent with the responses of the 12 monitored ions of TCDD standard. Quantitation is based on the observed responses at m/e 322 and m/e 334.

[c] See text for sample identity.

[d] Samples were not run due to large amounts of coextractives.

[e] Some or all of the sample was lost.

[f] Quantitation by external standard because of ^{13}C-TCDD carrier.

other laboratories (A, B) could be used for quantitative measurements with ions at m/e 322 and m/e 334, but there was no confirmation because interferences obscured many of the other monitored ions. The remaining laboratory used two different cleanup methods (D, E); in each case significant levels of coextractives inhibited quantitation or confirmation of the presence of TCDD.

Although the two interlaboratory studies had different objectives, together they serve to emphasize the critical relationship between the sample matrix, the cleanup, and the extraction method and the method of instrumental analysis. The most inefficient method in the fish study, Method D, was used to clean up the beef fat extracts in the first study. It is a relatively simple procedure involving base hydrolysis, acid partitioning, followed by two separate alumina chromatography steps. Clearly, laboratory H, using a very high-resolution mass spectrometer, was capable of accurately analyzing TCDD down to 10 pg/kg in beef fat extracts prepared by this method. On the other hand, laboratory E, which participated in both studies, had very erratic results when analyzing the same beef fat extracts. However, by using its own complex cleanup method, consisting of reagent-modified gravity-flow adsorption columns followed by two high-pressure liquid chromatography steps, laboratory F was able to produce extracts from fish that could be readily analyzed by gas chromatography/low resolution mass spectrometry.

CONCLUSIONS

Although environmental samples often consist of complex mixtures of chemicals, investigators can reliably detect trace quantities of individual chemicals by adhering to strict protocols for instrument calibration, method validation, and quality control. This paper has focused on one compound, 2,3,7,8-TCDD, in order to describe the considerable advantage inherent in the use of an isotope-labeled internal standard as a part of ion-monitoring mass spectrometry. However, if high recovery values can be obtained, then even in the absence of isotope-labeled internal standards, analysts can carry out multichemical analyses for closely related compounds with considerable accuracy and precision. For illustrations, see the work of Lamparski and Nestrick (1980) on the analysis of particulate matter for chlorinated dibenzo-p-dioxins.

Regulatory agencies are now attempting to develop analytical techniques for the study of a wide range of chemical compounds with different physical and chemical properties in industrial wastewaters and the like. In the interests of time and cost, these methods often involve minimal sample preparation and chromatographic separation, relying on the power of instruments such as mass spectrometers to differentiate between compounds. With traditional mass-spectrometric techniques, this may lead to inaccurate quantitation and even erroneous compound identification. However, recent developments in mass spectrometry, such as metastable ion analysis, show considerable potential for compound identification in complex mixtures and may therefore play an important role in the future development of environmental analysis.

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Standardization, Validation, and Quality Control for the Analytical Determinations

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The determination of several pollutants found simultaneously is a complex problem, which has not been completely explored from the chemical point of view. Although in the literature there is no lack of information about the environmental level of various pollutants, often the wide range of values in the published measurements limit the value of the data. Where the adoption of similar methods allows comparative evaluation of the results, variations in environmental concentration may remain, which one can reasonably refer to the type of the source, to geography and climate, or to the urban or industrial nature of the area studied.

The reliability of sampling and analysis methods is also complicated by the diversity of the matrices to be analyzed and of the type of measurement imposed by multiple contamination. Therefore, criteria and procedures that guarantee the availability of reliable and comparable analytical data must be clearly defined, even if this is only one stage in the solution of problems stemming from contamination by multiple pollutants. This is particularly imperative for the definition of environmental quality criteria on which prevention directives can be based.

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STANDARDIZATION

The quantitative measurement of contaminants is difficult because of the large number of potentially interfering compounds. Therefore, the principal need is for well-tested methods and appropriate standards or reference materials.

Calibration involves checking physical measurements against accepted standards. Standardization consists of determining the response function $S = f(c)$, where S is the measured net signal that is a function of the given substance concentration (c). One should carry out regression analysis with at least five different concentrations of the calibration standards measured in triplicate, which must include the expected concentration of the substance in the field sample. The standardization must be done under the same conditions as the measurement process. If testing indicates a controlled condition, the corresponding restrictions must be included in the standardization process.

Measurement methods in routine use are often compromises in terms of cost, analysis time, and experience of the laboratory staff; thus, each selected method should be exhaustively pretested for sources of error, and controls specified if necessary. Then the methods should be retested during the measurement process by periodic analyses of the blanks, standards, and "spiked" samples to monitor the corrective conditions intended to prevent anomalous results. Multicomponent analysis systems that determine the abundance and distribution of the chemical elements and products present in samples, as well as the degree of natural variability, prevent useless concern for a contamination level that may be natural to the system. The simultaneous analysis of many compounds reduces also the analytical error mainly related to the low number of sample preparations and offers the possibility of analyzing not only trace but also major compounds to present the results as ratios to one or more "stable" compounds.

Often several methods are used to measure the same sample, and the results can vary considerably from laboratory to laboratory. Therefore, definitive and reference methods are required. Many organizations are already involved in the standardization of methods, among others the American Society for Testing and Materials, the Association of Official Analytical Chemists, the International Organization for Standardization, Deutsche Industrie Norm, and Unificazione Industria Chimica. Reference methods are available, in principle, for most low molecular mass compounds. Individual laboratories must test the feasibility of such methods, however, to eliminate potential sources of systematic and random errors and to pay special attention to the matrix.

There must be support for the development of improved reference methods for the analysis of high molecular mass compounds. Interlaboratory comparisons frequently have shown a wide variation of results. This state of affairs would be considerably improved by the introduction of suitable reference materials, which should be produced from reference methods. The type of method would depend upon the material.

All reference materials are important, and any priority will of necessity be arbitrary. Nevertheless, a working order of priority would consider the importance of an accurate determination with particular reference to the toxicity of the products, the frequency of routine tests for which the specific reference material is applicable, and the general availability of instruments that do not provide absolute measurements.

In summary, the simplest procedure to obtain a true measurement probably is to calibrate the instruments by means of certified and appropriate reference materials. The nonavailability of appropriate reference materials has often given rise to poor interlaboratory comparability and lack of reliability. The large amount of expertise in this subject in several centers (see list on pp. 108-114 at the end of this paper) should facilitate the preparation of appropriate reference materials for the most important contaminants. However, because of possible differences between various manufacturers' preparations and between batches from the same manufacturer, a reference material characterized by a number of chemical and physical criteria would allow the manufacturers to adjust their test kits for quality control, thereby making them more reliable. Thus, there is a need for national reference materials and working standards linked to reference preparation.

The purity of a working standard should be documented, possible interferences determined, and the possible time-dependence of the composition of a standard measured to determine whether any change occurred during the analysis. There are three categories of possible reference materials and working standards:

1. products in the pure state, alone or in a mixture, which can be used for instrument calibration;

2. pure products added in known quantities to different matrices (their use would allow checking of instrumental analysis, when this may be carried out directly on the matrix, and of extraction and purification procedures in conditions similar to those of the real sample); and

3. real samples, with certification of the presumed levels of contaminants by analyses carried out with different reliable methods and, when possible, averages of analytical results from several qualified laboratories. The three classes are linked with the extent to which they are used. The materials of the first group are particularly suitable for instrument calibration, those of the second group to the evaluation of interference from matrices, and those of the third group to checking the recovery of the contaminants linked to the constituents of matrices. These last are the most valuable because they allow a complete verification of the analytical method under real conditions. Even if their absolute levels are only assumed, they are still of service to the individual laboratory because they allow comparison and correction of their own performance with that of specialized laboratories equipped with more advanced instruments and techniques.

VALIDATION

Specificity

The use of suitable reference materials allows one to check the general capacity of the laboratory, especially to define the measurement error and to carry out the regular calibration of the instruments. The validity of the analysis of the individual sample should, however, be guaranteed by checks that have been properly designed and adapted to the particular determination. Protocols, including all analytical procedures, should therefore be available to obtain reliable results.

This is true for both sampling and analysis. For example, the sampling of airborne substances must account for the interrelationship between exposure and possible absorption by the exposed subject as well as the facility of execution, reproducibility, and significance of the sampling, which are always very important parameters.

The analysis must be as appropriate and specific as the sampling. The term specific, which might assume a different significance when one refers to inorganic or organic analysis, must be clarified with some examples. Suspended airborne particulates in general include aerosols with sizes between 0.01 and 50-100 μm. The range of the granulometry and the variability of the chemical composition, however, make an evaluation of the "total" sample ineffectual. Therefore, specific measurements are necessary for the breathable fraction and for the particularly soluble toxic chemical species. A correct analytical measurement of a given species in its soluble chemical form, determined in its most easily assimilated granulometric fraction and not that excreted by the human body, is imperative. From this point of view the correlations obtained between pollutants such as lead and carbon monoxide or vanadium and sulfur dioxide, which have high correlation coefficients in well-defined urban situations, do not appear to be applicable. The correlation would allow us to move from the determination of the concentration of gaseous pollutants to that of corresponding heavy metals evaluated overall and not to the fraction adsorbed by the human body.

Such correlations are, however, useful for finding the origins of some pollutants. The same result is obtained with cluster analysis in metals. In fact, comparison of the different parameters shows that the closer the metals are, the more similar is their behavior and thus their origin. For example, trace aluminum, cadmium, copper, sodium, and potassium can be traced to foundries and iron, chromium, manganese, and cobalt to steel works.

Detecting the origin of the pollutant can also be accomplished by using labeled and isotopically differentiated compounds. This is a very specific technique, which is useful even for large-scale experiments.

Of course, specific analysis of constituents that are identified in their chemical and physical form is not always possible. The atmospheric particulate in the analysis of fibers is a valid example. Fibers can be elongated particles, as long as they are sufficiently small and have a length/diameter ratio greater than three. These include natural fibers, which are minerals (asbestos, hydrosilicates, and silicates) or organics (vegetable products such as cotton, linen, jute, etc., and animal products such as wool, silk, etc.), and artificial fibers, which are inorganics (sulfates, carbonates, etc.) or organics (acrylics, polyvinyls, polyesters, etc.).

Identifying the types of fiber would provide valuable information in the study of pollutants in the industrial environment, but the difficulties and uncertainties of the analytical determinations are considerable, and often the results obtained with the various methods are not in agreement. Only the combined use of optical and instrumental methods[2] yields reliable, although approximate, evaluations valid for fibers of known composition. The differences between operators, the complexity of the calibration necessary, the interference of the presence of unknown compounds, and the very small quantity of sample normally available are all factors. Thus, distinguishing the fibers according to their form is insufficient for the identification of the various types, in particular, of those most noxious for man.

The problem of determining 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD), which became of paramount importance in Italy after the 10 July 1976 accident in Seveso, provides a good example of specific organic determinations. 2,3,7,8-TCDD, one of the 22 possible isomers of TCDD, is the most toxic (Holmstedt, 1980), but there is no established minimum level for excluding the dangers of toxic effects, especially the carcinogenic and teratogenic effects on humans. Consequently, we should use determination methods that are specific and sensitive at a level of parts per trillion (ppt). However, the diversity of the matrices, the presence of interfering contaminants, and the necessity of separating 2,3,7,8-TCDD from the remaining 21 possible isomers make determinations of this isomer in environmental samples particularly difficult at the ppt level.

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[2] Optical methods involve use of microscopes, such as the polarizing microscope, chromatic dispersion microscope, phase contrast microscope, ultra-microscope, or reflected light microscope. Instrumental methods include x-ray diffraction, microprobe analysis, single crystal electron diffraction, analytical electronic microscopy, and infrared spectrometry.

Many isomers of TCDD have gas chromatographic retention times close to that of the 2,3,7,8-TCDD isomer (Buser and Rappe, 1980). Therefore, gas chromatography can separate them only by the use of capillary columns covered with different liquid phases of such lengths (~50 m) that analysis times are long and routine application is awkward.

Thus, the determination of 2,3,7,8-TCDD at the ppt level in environmental samples requires instruments of high specificity and sensitivity. At present, there are three categories of analytical methods that can satisfy these requirements, based on the gas chromatography/mass spectrometry technique:

1. purification and chemical separation at a high degree of specificity through use of an analytical apparatus having a low resolving power;

2. purification and specific chemical separation through use of an analytical apparatus having a high resolving power; and

3. purification, specific chemical separation, and instrumental separation based on high performance liquid chromatography through use of a detecting apparatus having a high resolving power and high sensitivity.

The use of high resolution instruments distinguishes the second and third methods from the first. The use of analytical instruments having a low resolution is valid only in a preliminary screening phase (unless the sample had been subjected to an appropriately tested purification of very high specificity), which must be followed by checking the positive samples with a high resolution apparatus. The third method seems the most suitable for lowering the detection limit and probably is the only one capable, in routine conditions, of supplying specific measurements for the single TCDD isomer. In fact, one can separate the 22 isomers of TCDD by using high performance liquid chromatography in the combination reverse phase and normal phase; using it at the end of the specific purification cycle for the particular environmental matrix would improve the quality of the extract and thus yield a higher signal-to-noise ratio in the mass spectrometric measurement. There would be an advantage in using higher gains for the output signal of the spectrometer, which would lower the limit of sensitivity.

In summary, the following procedure should yield a sure quantitative measurement of the 2,3,7,8-TCDD present at a ppt level in environmental samples of any origin:

1. extraction, purification, and separation as per the

specific method for the particular matrix involved;

2. high performance liquid chromatography (HPLC) in reverse phase, with collection of the sample at the 2,3,7,8-TCDD elution time;

3. HPLC in normal phase, with collection of the sample at the 2,3,7,8-TCDD elution time.

4. gas chromatography with capillary columns;

5. simultaneous measurement with a high resolution mass spectrometer (resolution power ∿10,000) of the signal of masses 320 and 322 for native TCDD and of the mass corresponding to the most intense molecular ion of the labeled TCDD, if added (greater specificity would derive from the measurement of a third ion for the native TCDD--the molecular ion at mass-charge ratio 324 or the fragment ion at 257--and from the measurement of the most intense molecular ion of a second, differently labeled TCDD); and

6. signal-to-noise ratio greater than or equal to three.

The certainty of the identification of the 2,3,7,8-TCDD should be based on the following determinations:

1. retention time in gas chromatography equal to that of the standard 2,3,7,8-TCDD;

2. abundance of the isotopic molecular ions and fragments with values equal to those found for the standards;

3. intensity ratio of the signals for the molecular ions of the labeled TCDD of the sample equal to that expected from their concentrations or equal to that obtained by direct analysis of the initial solution of the labeled TCDD of the sample;

4. repetition of the entire procedure on at least 10% of the samples showing positive results; and

5. analysis of control samples free of 2,3,7,8-TCDD (blanks) treated in parallel with the samples examined.

Other Factors

In addition to the high specificity needed for analysis of the most toxic contaminants, other factors can invalidate the analysis. Before the invention of sufficiently sensitive methods, experts believed that people did not contain many contaminants in their bodies, except in cases of accidental poisoning. The development of sensitive analytical techniques should reveal the common occurrence of traces of contaminants in humans and their environment, particularly in food. However, reliable trace analyses depend primarily on accurately determined blanks and only secondarily on the accuracy of the method itself.

Often analyses of many contaminants are unreliable because of an unfamiliarity with the extent, sources, and control of contamination during sample collecting, handling, and analysis. Consequently, many published data contain gross positive errors, and the error noise in concentration data determined at trace levels obscures the meaning of most work. At trace levels, the analyst must know with certainty the magnitude of the contribution of the contaminants under investigation from each reagent, from air exposure, and from laboratory ware. Without appropriate precautions, sophisticated analytical instruments are ineffective. Thus, widespread use of clean laboratory practices is imperative. All the sample pretreatment operations, which may include sieving, blending, crushing, drying, dissolution, dilution, filtration, and addition of preservatives, should be documented so that the treatment used can be duplicated. Procedures should use controls and calibrations to prevent random and systematic error and provide high recovery with minimum contamination, and the number of steps in the procedure should be kept to a minimum in order to reduce the possibility of errors.

Precision and accuracy of measurement give a clear indication of the quality of the analysis. Where measurements are conducted with working standards, excessive measurement variability indicates probable uncontrolled systematic errors. If precautions have been taken to eliminate the systematic errors, the remaining fluctuations are considered random and will determine the experimental precision. The absolute signal variability (σ) is defined by the standard deviation in the estimated net signal (Sx). This quantity should be based on at least 10 observations (Crummett et al., 1980).

The relative variability of analytical measurements increases as the substance concentration decreases. There are three regions of reliability--the levels of determination, detection, and uncertain detection--in descending order of reliability. The limit of detection is the lowest concentration of a substance that the analytical process can reliably detect. The observed signal (St) is the sum of the instrumental response (Sx) due to the presence of the substance (x) in the sample, plus a response signal (Sb) due to the background contribution (e.g., St = Sx + Sb). The limit of detection should be located at least at 3σ above the blank signal Sb (e.g., $St \geq Sb + 3\sigma$). A value of three is considered minimal, as it implies the risk of false positive decisions; a more conservative value (e.g., six) will decrease the risk of false results (Crummet _et_ _al_., 1980). The measurements are unreliable when they produce an excessive number of false positives or false negatives.

Initial positive results on actual field samples can be evaluated by repeated analyses of subsamples from the same field samples. Agreement between replicate analyses above the limit of detection increases confidence in the measurement. However, final data are not validated until two or more independent methods provide consistent results.

The confirmation procedure should be highly selective and based on analytical principles for analytical conditions different from those used in the initial method. Thus, one gas chromatography-mass spectrometry method may be validated by another that differs in the chromatographic conditions, ionization technique, or detection system. The region for quantitation should be above the limit of detection. The recommended minimum value is 10_σ, e.g., $St \geq Sb + 10\sigma$; signals less than 36 should be reported as not detected (Crummett _et_ _al_., 1980).

The recovery rate of a method is usually derived from the measurement of spiked blanks containing known added concentrations of the substance. Added to a blank sample, the substance may behave differently (typically showing higher recovery) from that in the field sample. Care should be taken when spiking the sample with an appropriate tracer. One cannot affirm that the recovery based on a sample spiked with a labeled compound has a certain percentage value until an assessment has been made of the conditions and solvents used for the addition of the tracer. One must facilitate the absorption of the internal standard by the material examined and endeavor to submit the tracer to extraction conditions that are as close as possible to those of the endogenous product.

Whenever possible, testing should include experiments on homogeneous working standards containing known amounts of a naturally incorporated substance. Unfortunately, the frequent lack of such samples is an important limitation in trace analysis. As the recovery rate falls, the measurement process becomes more dependent on the knowledge of the precision of the recovery at that concentration. It is preferable to obtain reproducible recoveries rather than high, but variable, ones. Great variations increase the likelihood of an external unforeseen cause, which may render the procedure uncontrollable. Low recovery methods may be satisfactory in the region of quantitation only if the accuracy and precision are established. Recoveries of less than 50% should be considered unreliable.

Other useful precautions can ensure results close to a true value. Because preserving biological or vegetable tissues in unsealed containers in freezers may cause partial dehydration, the samples must be previously weighed; desiccation may prevent an exact correlation of the concentration of the compound with that of the original tissue. Care should be taken during the extraction phase, because different types of matrix could, at a trace level, produce drastically different results. Thus, methods tested for TCDD analysis for vegetable tissues having a high water content reveal definitely inferior and scarcely reproducible results in the case of cereal samples; the same problem exists when the type of soil or tissue varies. A thorough examination of the extraction phase for different samples is therefore important, as well as the selection of the volume and number of extractions and the most suitable apparatus for the extraction.

In summary, when decisions regarding the presence of contaminants are based on results of compositions near or below levels measured by conventional techniques, the analyses are subject to numerous difficulties including interferences. A strategy is needed to reduce the error. This includes minimizing the complexity of the procedure and evaluating the experimental variable, thus reducing the opportunities for error that may arise when the measurement process is sensitive to small changes in operation. The reliability of analytical information depends upon the rigorous fulfillment of all the requirements stated in a well-defined analytical protocol, including confirmation and validation of the measurements. If doubts arise, additional analyses should be performed with other methods. Unusually high or low results should be validated by analysis of a duplicate subsample by the same method and a third subsample by a different method. Finally, accurate data are far more likely to be obtained when supported by the use of calibration and working standards. Field blanks and field samples should also be periodically analyzed.

QUALITY CONTROL

The term quality control usually refers to a procedure by which samples of known composition are periodically analyzed and the results statistically evaluated to determine the accuracy and precision, at least. Control samples, as similar as possible to the unknown material, should be randomly injected in the different series of analyses in order to measure them under the same conditions as the unknown material. Double blind samples, if not identifiable, could also be periodically analyzed.

Interlaboratory comparison of homogenized subsamples may indicate serious discrepancies due to undetected errors. Youden's correlation technique (Youden, 1960) could be used in order to distinguish between random errors and laboratory bias.

The basic objective of a quality control program is to ensure constant reliability of the results. Periodic checking and calibration of equipment should result in an exact knowledge of the precision and accuracy of the analyses and provide an incentive for additional improvements in the measurements. A good quality-control system offers the opportunity for improving not only the analytical capability of the laboratory, but also the aggregate performance of its personnel. As it is difficult to teach laboratory personnel how to eliminate errors, a quality control system should also include control of the errors within the responsibility of the laboratory and procedures for recognizing variability. Thus, the quality control should extend from the collection of samples to the reporting of results. In this sense, the term quality control could be replaced by the terms quality assurance, proficiency testing, or performance evaluation.

Errors and variability in the analysis can be introduced at several stages. They could include the choice of the sample, method of collection, sample identification, storage containers, transport systems, subsampling, analytical procedure, poor specificity or inadequate sensitivity of the apparatus used, calculations, and reporting results. One should evaluate all these steps and propose adequate solutions for eliminating the largest errors.

Many minor aspects of the analytical system are often not considered, and the variability introduced at these stages is frequently underestimated. Some examples are contaminant-contaminant interactions, the laboratory conditions (i.e., noise levels, temperature, humidity, cleanliness, etc.), a constant high work load of the laboratory staff, lack of involvement of the senior staff members, and difficult relationships between various members of the staff.

The variability of a method is usually established under optimal conditions by the most skilled operator working with sufficient time in an ideal environment and using an appropriate apparatus for specific measurement, checked reagents, and homogeneous samples. Although the variation measured under such optimal conditions is important for evaluating a method, knowledge of the variation of a method determined in routine conditions by an average operator on an average day is also important. The quality control system therefore must provide accurate assessment of both routine and optimal condition variances.

Because most analytical errors occur within individual laboratories rather than between experienced laboratories, adequate analytical performances would best be achieved by paying increased attention to internal quality control and by making a periodic review of the procedures. An internal quality control should include provision of representative samples and controls, use of replicate samples, and correction of departures from standards of quality.

Difficulties in the external quality control could derive from the methods chosen, which may not be homogeneous. In principle, the method must be sufficiently sensitive, precise over the entire range of concentrations, and without interference from other compounds. In practice the choice of methods is often based on the availability of instruments, on their cost, and on the experience of the laboratory staff. Therefore, results obtained by reliable methods are compared with others less reliable. To avoid this inconvenience the results obtained by reliable methods could be taken as reference values. Mass spectrometric methods are generally considered to be the most reliable and specific, if operated in high resolution and multidetection mode, but they require access to expensive equipment and skilled operators. The combination of internal and external quality controls is an effective means of improving and sustaining the quality of determinations. Therefore, regular quality control programs will eliminate at least one uncertainty in decision-making--the reliability of the results obtained.

CONCLUSIONS

Precise and accurate measurements depend on the availability of proven methods, proper equipment, and individual skill. No measurement program should lack a well-designed measurement process established among the analysts, the statisticians, and the scientists who will use the data.

Accuracy is supported by the use of reference materials and participation in interlaboratory comparison activities. Performance testing, based on the use of working standards, is needed to monitor the recovery and variability in measuring samples and blanks. A complete report should provide sufficient and pertinent information on the sample, analytical procedure, instrumental measurements, and data treatment as well as on the possible interferences that can arise at any stage in the analytical process. Accurate chemical analysis cannot be based only on the performance of sophisticated and sensitive instruments. Thus, modern analytical chemistry requires detailed protocols on the measurement system, sensitive and specific methods, permanent validation process, and systematic use of quality control procedures, which ensure the validity of the overall analytical measurement process.

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Organizations Marketing Various Types of Reference Materials

L'Air Liquide
Paris 7e, France

Air Products
Stoke-on Trent, United Kingdom

Aldrich Chemical Company
Milwaukee, Wis., U.S.A.

All Union Scientific Research
Institute of Metrology
Sverdlovsk Branch, U.S.S.R.

Alpha Metals, Inc.
Jersey City, N.J., U.S.A.

Aluminum Company of America
New Kensington, Pa., U.S.A.

American Petroleum Institute
Pittsburgh, Pa., U.S.A.

Analabs, Inc.
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Billiton
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Bundesanstalt Fuer Materialpruefung
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Bureau of Analysed Samples, Ltd.
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New England Nuclear Corp.
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PART II: ENVIRONMENTAL INTERACTIONS

Role of Chemical Interactions in the Assessment of Multichemical Contamination

Morton Lippmann and Paul J. Lioy[1]

Chemicals released into the air, surface waters, and soil will generally react with other chemicals in those media. The resulting products will frequently react with other chemicals, and complex series of reactions may continue along extended physical transport pathways before the materials find semipermanent storage sites in terrestrial soils or aquatic sediments. The pathways and transit times for some pollutant chemicals are relatively simple and reasonably well-understood. For example, carbon monoxide (CO) can only react with oxygen (O_2) to form carbon dioxide (CO_2) or be taken up and metabolized by the biosphere. Its atmospheric oxidation rates and residence times have been described (National Academy of Sciences, 1977a; U.S. Environmental Protection Agency, 1979).

Another gas-phase combustion product, sulfur dioxide (SO_2), has a much more complex series of atmospheric interactions. It undergoes oxidation to sulfur trioxide (SO_3), via reactions with free radicals such as the hydroxyl, the hydroperoxy, and the methoxy radicals (HO, HO_2, and CH_3O_2), by reaction with ozone (O_3) and alkenes, by reactions after dissolution into aqueous droplets, and by reactions on the surfaces of graphite and soot particles. Reactions within droplets and on surfaces depend greatly on their compositions. The sulfur trioxide formed in the gas phase reacts in milliseconds within water vapor to sulfuric acid (H_2SO_4), a low-vapor-pressure droplet aerosol that is considerably more toxic than sulfur dioxide. As an aerosol, sulfuric acid has a longer residence time within the atmosphere than does sulfur dioxide. Thus, the transformation, by whatever route, results in a greater potential for health effects in downwind populations. On the other hand, further chemical reactions take place, primarily neutralization of the acid with ammonia (NH_3) to produce ammonium sulfate [$(NH_4)_2SO_4$], which greatly reduces its potential health effects.

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In this paper, we shall discuss further the pathways and reaction rates for this complex series of events within the atmosphere as prime examples of chemical interaction. Another example is the photochemical sequence of reactions that lead to the formation of ozone. In this case, the toxic endproduct is highly reactive in comparison to the primary precursor pollutants, i.e., NO_x [nitric oxide (NO) plus nitrogen dioxide (NO_2)] and hydrocarbons, and in comparison to its reaction products. In each of our two examples the most toxic species, i.e., sulfuric acid and ozone, can be persistent only when the atmosphere is enriched with their precursors and the temperature and radiant conditions are right for their continued formation.

Chemical interactions affecting human exposure and environmental quality also occur in surface waters and soils. However, these situations are usually much more site-specific than the two examples selected for discussion, and there are few situations where there is sufficient information available for the development of a good case history. Thus, we will limit this presentation to atmospheric chemical interactions where there is a reasonably complete body of relevant data and which affect a very large number of people.

PHOTOCHEMICAL SMOG FORMATION

The formation of ozone and other photochemical smog products, including high concentrations of fine particles and a variety of eye irritants, is a complex process. It takes place through a series of chemical reactions and depends upon the presence of sunlight and the right mixture of precursor pollutants. Smog episodes are often observed on hot sunny days, and the concentrations of products usually increase as the air mass travels from dense urban centers to areas downwind. Most of our knowledge of ozone formation mechanisms comes from laboratory chamber research (Altshuller and Bufalini, 1971; Leighton, 1961; National Academy of Sciences, 1976). These investigations used various hydrocarbon-nitrogen oxide-air-sunlight mixtures, and, although the details of each experiment differed, they all produced temporal patterns of pollutant concentrations similar to that shown in Figure 1.

In the first phase, there is the conversion of nitric oxide to nitrogen dioxide, and the second phase finally produces ozone. Mixing and ventilation can affect the buildup of pollutants within the atmosphere as well as the transport of pollutants and their reaction products to areas downwind of the precursor sources (Lioy and Samson, 1979; Vukovich, 1977). As summarized in the ozone/oxidant criteria document (U.S. Environmental Protection Agency, 1978), the net result of the ozone production scheme can be represented by the following main reactions:

$$NO_2 + h\nu \rightarrow NO + O \qquad (1)$$

$$O + O_2 + M \rightarrow O_3 + M \qquad (2)$$

$$O_3 + NO \rightarrow NO_2 + O_2 \qquad (3)$$

$$XO_2 + NO \rightarrow NO_2 + XO \qquad (4)$$

$$2NO + O_3 \rightarrow 2NO_2 \qquad (5)$$

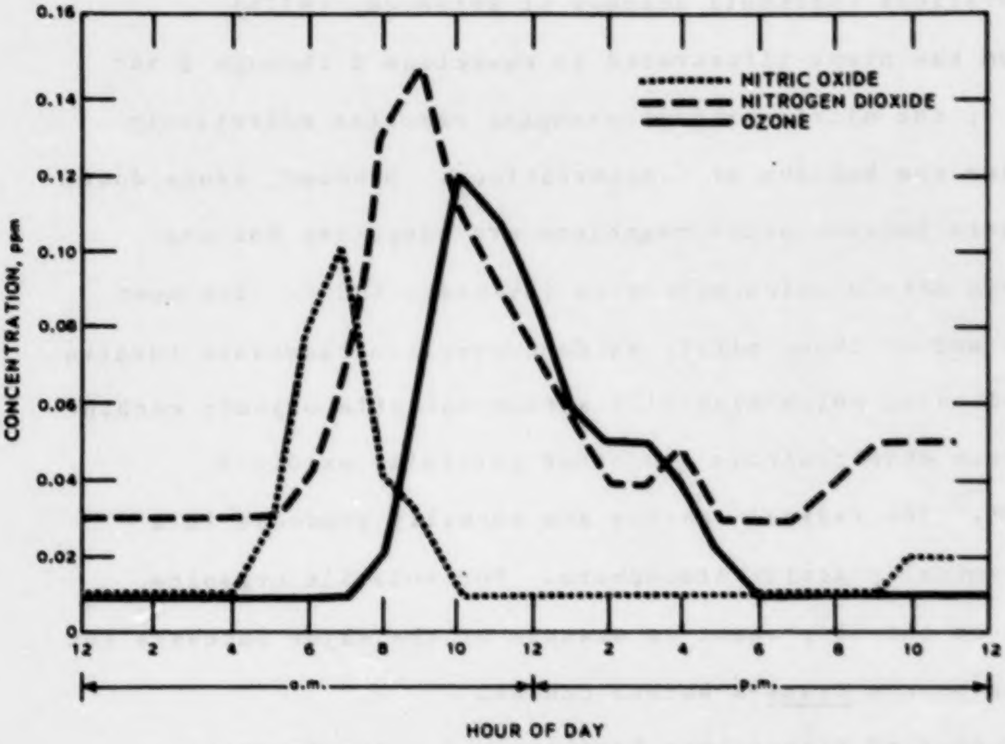


FIGURE 1. Diurnal variation of nitric oxide, nitrogen dioxide, and ozone concentrations in Los Angeles, 19 July 1965. (Reprinted from Air Quality Criteria for Ozone and Other Photochemical Oxidants, National Academy of Sciences, 1977)

where X is equivalent to hydrogen or a free radical.

There are many pathways within photochemical processes, and more research is needed to determine the products of individual organic or inorganic reactions and the concentrations of the free radical products. At present, many products cannot be determined directly; they may be a result of thermochemical considerations (National Academy of Sciences, 1977b).

From the steps illustrated in equations 1 through 3 and Figure 2, the nitric-oxide-scavenging reaction effectively precludes the buildup of concentrations. However, ozone does accumulate because other reactions are competing for the available nitric oxide molecules (Graedel, 1980). The most significant of these nitric oxide conversion reactions involve free radicals, which also will attack volatile organic carbon to produce more radicals and other partially oxidized products. The radical species are normally produced in a photochemically active atmosphere. For volatile organics, Figures 3a and 3b present an example of the major pathways for reaction by the trans-2-butene scheme.

A number of researchers have adopted a steady-state hypothesis for ozone accumulation using equations 1 through 3. The solution of the equations yields the following:

$$[O_3] = \frac{k_1 \, [NO_2]}{K_3 \, [NO]}$$

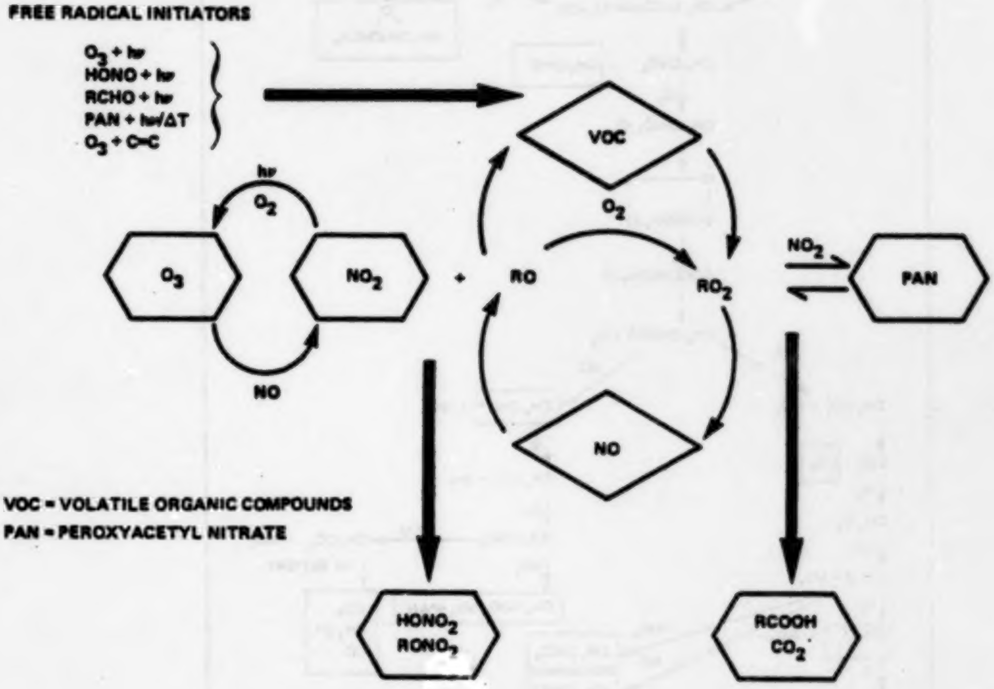
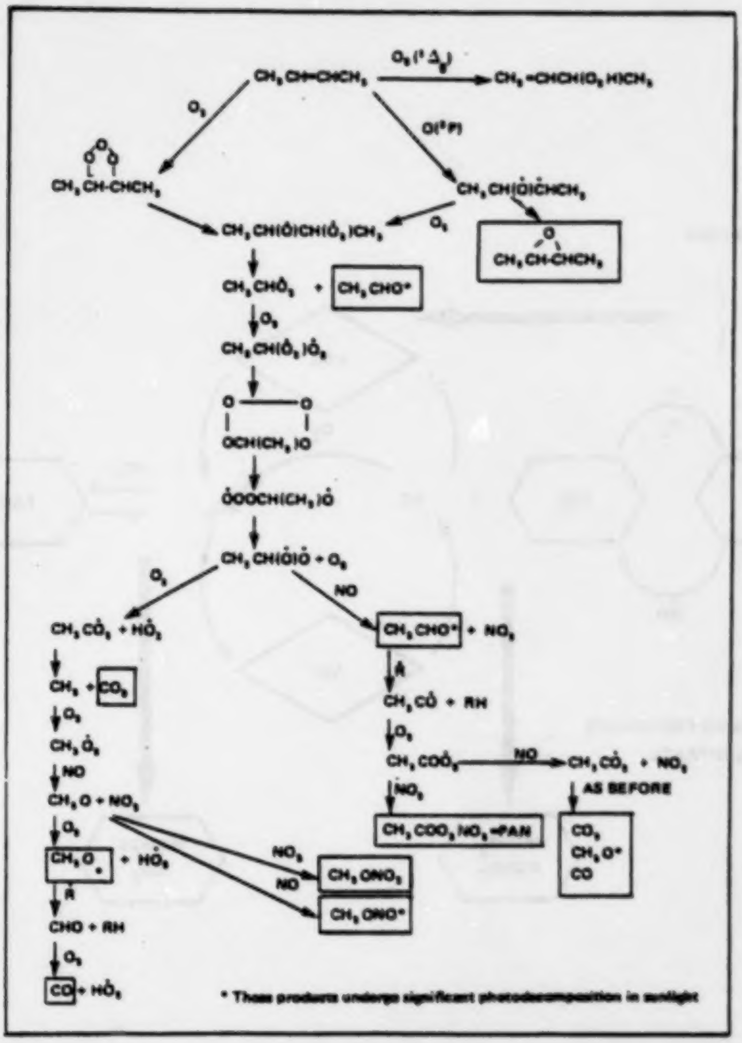

FREE RADICAL INITIATORS
O_3 + hν
HONO + hν
RCHO + hν
PAN + hν/ΔT
O_3 + C=C
hν
O_2
O_3
NO_2
NO
+
RO
VOC
O_2
RO_2
NO_2
PAN
NO
$HONO_2$
$RONO_2$
RCOOH
CO_2
VOC = VOLATILE ORGANIC COMPOUNDS
PAN = PEROXYACETYL NITRATE

FIGURE 2. Schematic of the polluted atmospheric photo-oxidation cycle. See text for details. (From *Air Quality Criteria for Particulate Matter and Sulfur Oxides*, U.S. Environmental Protection Agency, 1981)



* These products undergo significant photodecomposition in sunlight

FIGURE 3a. The major reaction paths for the degradation of *trans*-2-butene in an irradiated NO_x-polluted atmosphere. (Reprinted from Air Quality Criteria for Ozone and Other Photochemical Oxidants, U.S. Environmental Protection Agency, 1978)

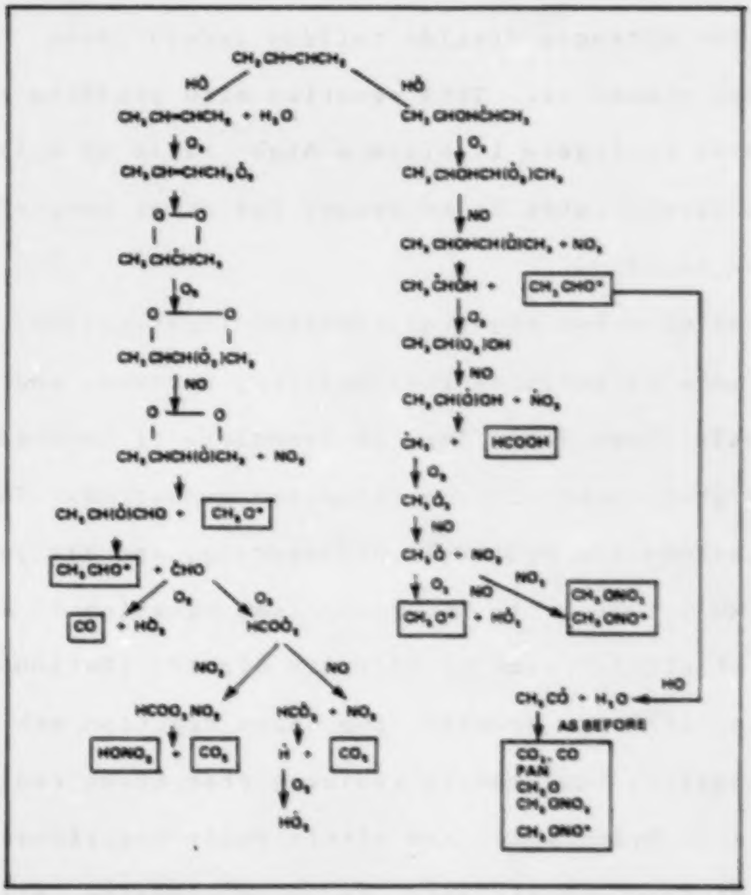


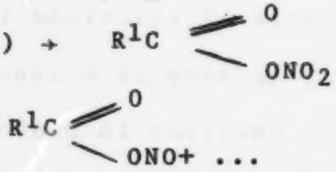
FIGURE 3b. Continuation of the major reaction paths for the degradation of trans-2-butene in an irradiated NO_x- polluted atmosphere. (Reprinted from Air Quality Criteria for Ozone and Other Photochemical Oxidants, U.S. Environmental Protection Agency, 1978)

where K_3 is the rate constant for reactions 2 and 3, and k_1 is the dissociation constant for nitrogen dioxide. Atmospheric studies have essentially verified this equation within the stochastic bounds of a turbulent atmosphere and illustrate the necessity for nitrogen dioxide buildup before ozone accumulation commences. This equation also predicts the ozone buildup curve in Figure 1, since a high ratio of nitrogen dioxide to nitric oxide is necessary for ozone concentrations to increase rapidly.

In terms of other chemical-chemical interactions, advances have been made in defining the identity, sources, and role of free radicals produced during the reactions of hydrocarbons and NO_x in the photochemical-smog formation mechanism. These radicals include the hydroxyl, hydroperoxy, and alkoperoxy radicals (RO_2), which are important (see equation 4) in the oxidation of nitric oxide to nitrogen dioxide (National Academy of Sciences, 1977b). However, the ozone reaction schemes used for photochemical smog models indicate that these radicals also participate in hydrocarbon and nitric oxide reactions that produce aldehydes, nitric acid, hydrogen peroxide, nitrous acid, peroxyactylnitrate (PAN), and more radicals (Dimitriades and Altshuller, 1977; Graedel, 1980; Winter et al., 1979). Because of the complex mixtures of hydrocarbons in the atmosphere, each reaction has a different rate, which will result in varying yields of these products. Some examples of the nitrate formation mechanisms are shown in Tables 1a and 1b;

TABLE 1a

Reactions Potentially Involved in Nitrate Formation

Species	dNO_2/dt, or Rate Constant (ppm/min)[a]
Nitrogen oxides	
1. $O_3 + NO \rightarrow NO_2 + O_2$	2.7×10^{-2}
2. $O + M + NO \rightarrow NO_2 + M^b$	--
3. $RO_2 + NO \rightarrow NO_2 + RO$	2.5×10^{-3}
4. $O_3 + NO_2 \rightarrow NO_3 + O_2$	-4.0×10^{-4}
5. $NO_3 + NO_2 \rightarrow N_2O_5$	-1.0 to -23×10^{-4}
Volatile acids	
6. $N_2O_5 + H_2O \rightarrow 2HONO_2$	2×10^{-5}
7. $HO + NO_2 + M \rightarrow HONO_2 + M^b$	-10^{-5}
8. $NO + NO_2 + H_2O \rightarrow 2HONO$	--
9. $HOSO_2O + NO \rightarrow HOSO_2\,ONO$ $+ H_2O \rightarrow H_2SO_4 + HONO$	--
10. $HOSO_2O + NO_2 \rightarrow HOSO_2\,ONO_2$ $+ H_2O \rightarrow H_2SO_4 + HONO_2$	--
Gaseous nitrates	
11. $NH_3 + HONO_2 \rightarrow NH_4NO_3$	$\sim 10^{-6}$
12. $RO_2 + \begin{pmatrix} N_2O_5 \\ NO_2 \end{pmatrix} \rightarrow$ 	-10^{-3}

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[a] Typical for smog reactant concentrations in the first hour of reaction (e.g., Calvert and McQuigg, 1975).

[b] $M = 1$ atm N_2.

TABLE 1b

Aqueous Reactions of Nitrogen Oxides

13. $N_2O_5 + H_2O(\ell) \rightarrow 2H^+ + NO_3^-$
14. $NO + NO_2 + H_2O(\ell) \rightarrow H^+ + NO_2^-$
15. $2NO_2 + H_2O(\ell) \rightleftarrows H^+ + NO_3^- + HONO$
    $HONO + OH^- \rightarrow H_2O + NO_2^-$
16. $2NO_2^- + O_2(aq) \rightarrow 2NO_3^-$
17. $NO_2^- + O_3 \rightarrow (aq)NO_3^- + O_2$
18. $2NO_2 + H_2SO_4 \rightleftarrows HNOSO_4 + HNO_3$
    $HNOSO_4 + H_2O(\ell) \rightleftarrows HNO_2 + H_2SO_4$
    $3HNO_2 \rightleftarrows HNO_3 + 2NO + H_2O$
19. $RONO_2 + H_2O(\ell) \rightarrow H^+ + NO_2^- + R^1OH$

of the gas-phase reactions, numbers 6 and 7 are the most important in nitric-acid vapor formation.

Clearly, free radical species, especially the hydroxyl radical, play an important role in producing ozone and other potentially irritating gaseous and particulate species. Moreover, the supply of nitrogen oxides is a limiting factor in the entire photochemical-smog production mechanism since, in urban areas, the concentration of hydroxyl radical is dependent upon the reaction $NO + HO_2 \rightarrow NO_2 + HO$.

The details of the free radical reactions remain uncertain with respect to (a) the actual rate constants for all the reactions of hydroperoxy radical and alkoperoxy radicals; (b) the reaction sequence following hydroxyl-radical addition to olefins; and (c) the details of reactions involving alkyl and alkoxy radicals (National Academy of Sciences, 1977b). Table 2 lists compounds typically observed in photochemical smog, and Table 3 lists suspected compounds. These products will participate in the generation of more radicals and other stable products within various reaction chains. Furthermore, if other reactive chemicals, such as sulfur dioxide, are present, other reaction chains can also be initiated. Ultimately, relatively stable products such as sulfuric acid, nitric acid, and organic aerosols are produced and remain for some time within the atmosphere.

The organic aerosols include compounds containing aldehyde, carboxyl, and other functional groups (Hidy *et al*., 1980). Chamber studies have demonstrated olefinic reactions with ozone

TABLE 2

Compounds Observed in Photochemical Smog

Compound	Formula	Typical Conc.(ppm)	Maximal Conc.(ppm)	Reference
Ozone	O_3	0.1	0.6	U.S. Environmental Protection Agency, 1978
Peroxyactylnitrate	$CH_3COO_2NO_2$	0.005	0.2	U.S. Environemntal Protection Agency, 1978
Hydrogen peroxide	H_2O_2	--	0.18	Bufalini et al., 1972
Formaldehyde	CH_2O	0.04	0.16	Altshuller and McPherson, 1963
Higher aldehydes	RCHO	--	0.36	Renzetti and Bryan, 1961
Acrolein	CH_2CHCHO	--	0.11	Renzetti and Bryan, 1961
Formic acid	HCOOH	--	0.05	Hanst et al., 1974

TABLE 3

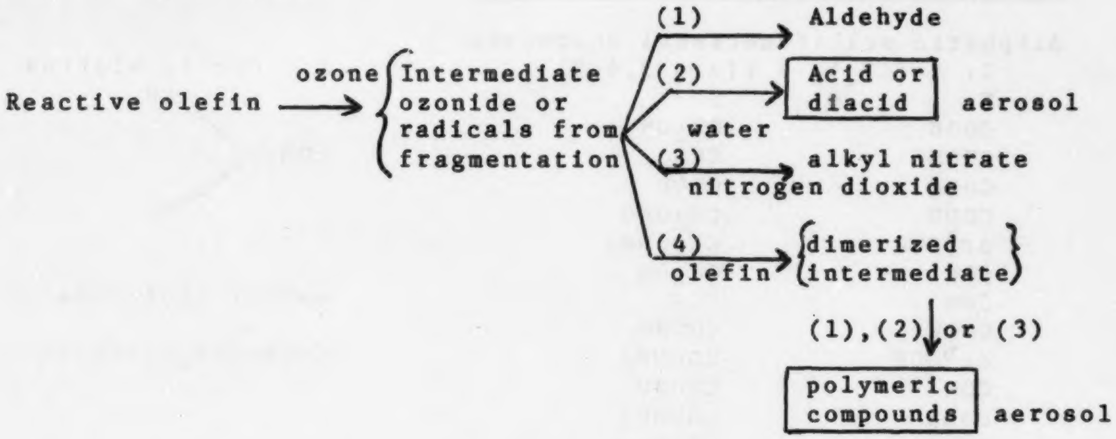
Compounds That May Be Formed in Photochemical Smog

Compound	Formula	Possible Synthesis	Reference
Peroxybenzoylnitrate	$C_6H_5COO_2NO_2$	$\phi COO_2 + NO_2$	Heuss and Glasson, 1968
Nitric acid	$HONO_2$	$NO_2 + OH$	Hanst *et al*., 1974
		$N_2O_5 + H_2O$	Morris and Niki, 1973
Organic hydroperoxides	ROOH	$RO_2 + HO_2$	Demerjian *et al*., 1974
Organic peracids	$RCOO_2H$	$RCOO_2 + HO_2$	Demerjian *et al*., 1974
Organic peroxynitrates	RO_2NO_2	$RO_2 + NO_2 + M^a$	Demerjian *et al*., 1974
Ozonides	O_3-olefin	O_3 + olefin + M^a	Atkinson *et al*., 1973
Ketene	CH_2CO	O_3 + olefin	McAfee *et al*., 1974
Nitrous acid	HONO	OH + NO	Cox *et al*., 1976; Atkinson *et al*., 1975
Pernitric acid	HO_2NO_2	$NO_2 + HO_2 + M^a$	Levine *et al*., 1977; Niki *et al*., 1977; Graham *et al*., 1977
Pernitrous acid	HO_2NO	$NO + HO_2 + M^a$	Cox and Derwent, 1975
Organic nitrates	$RONO_2$	$RO + NO_2$	Darnall *et al*., 1976
		$RO_2 + NO$	Darnall *et al*.,1976

---

[a] M represents any molecule that takes part in the three-body process.

that produce low-vapor-pressure dicarboxylic and monocarboxylic acids and organic nitrates. The following is a sketch of the reaction pathways described by O'Brien _et al_. (1975):

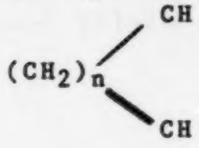

Reactive olefin
ozone
Intermediate ozonide or radicals from fragmentation
(1)
Aldehyde
(2)
Acid or diacid
aerosol
water
(3)
alkyl nitrate
nitrogen dioxide
(4)
olefin
dimerized intermediate
(1),(2) or (3)
polymeric compounds
aerosol

Many secondary organic aerosol compounds that have been identified in the atmosphere have been suspected to comprise a significant portion of the urban fine particle ($D_{50} = < 2.5\ \mu m$) fraction in photochemical smog.

In the Pasadena California Air Characterization Study (ACHEX), Hidy _et al_. (1980) observed a number of secondary organic aerosol compounds, as presented in Table 4. Of course, these are not all of the possible compounds, but the list gives an idea of the complexity of the organic-aerosol generation process in photochemical smog. Size-distribution measurements completed during this study showed that a significant fraction of these aerosols had aerodynamic diameters of less than 0.5 µm. Unfortunately, there is apparently no direct relationship

TABLE 4

Secondary Organic Aerosols[a]

Compounds Identified		Possible Gas-Phase Hydrocarbon Precursors
Aliphatic multifunctional compounds		
1. $X-(CH_2)_n - Y (n = 3,4,5)$		1. Cyclic olefins
X	Y	
COOH	CH_2OH	
COOH	COH	
COOH	COOH	
COOH	CH_2ONO	
or[b]COH	CH_2ONO_2	
COH	CH_2OH	
COH	COH	and/or diolefins
COOH	COONO	
or[b]COH	$COONO_2$	$\rangle C=CH-(CH_2)_n-CH=C\langle$
COH	COONO	
COOH	$COONO_2$	
COOH	CH_2ONO_2	
2. Others:		2. Not known; possibly from aromatic ring cleavage
$CH_2OH-CH=C(COOH)-CHO$		
$CH_2OH-CH_2-CH=C(COOH)-CHO$		
$CHO-CH=CH-CH(CH_3)CHO$		
$CH_2OH-CH=CH-CH=C(CH_3)CHO$		
$C_5H_8O_3$ isomers[b]		
Nitrocresols		
$C_6H_6O_2$ isomers[b]		
Aromatic monofunctional compounds		
3. $C_6H_5-(CH_2)_n-COOH (n=0,1,2,3)$		3. Alkenylbenzenes $C_6H_5-(CH_2)n-CH=CHR$; also toluene for C_6H_5COOH
4. $C_6H_5-CH_2OH$		4. Toluene, styrene, other monoalkylbenzenes?
C_6H_5CHO		
Hydroxynitrobenzyl alcohol		
Terpene-derived oxygenates		
5. Pinonic acid		5. α-Pinene
Pinic acid		
Norpinonic acid		
6. Isomers of pinonic acid:[b]		6. Other terpenes?
$C_9H_{14}O_2$ isomers		
$C_{10}H_{14}O_3$ isomers		
$C_{10}H_{16}O_2$ isomers		

---

[a] Compounds identified at West Covina, California, 24 July 1974. (From National Academy of Sciences, 1976)

[b] Isomers not resolved by mass spectrometry.

between gas-phase photochemical reactivity of the various precursors and the quantity of aerosol formed. It is probably a complex function of the rate of reaction of ozone, oxygen atoms, and hydroxyl radicals, hydroperoxy radicals, and other radicals. In addition, the nature of a given hydrocarbon present will be of significance in determining how easily an aerosol is formed. This is related to the nature of the products formed, the product volatility, and the aerosol-formation-ability index. Given all these facts concerning the role of photochemistry in the production of photochemical smog, however, ozone accumulation downwind appears to occur in areas devoid of major sources of hydrocarbons and nitrogen oxides (Dimitriades and Altshuller, 1977). Some of the possible explanations include transport from urban areas, local generation of urban ozone precursors, local generation of ozone from anthropogenic and nature precursors, and injection of stratospheric ozone.

What are the conditions for chemical-chemical interactions in the atmosphere when photochemistry cannot occur, i.e., at night? The material produced by photochemical reactions during the daytime mixes to great heights in the troposphere (assuming a mean mixing height of 1500 m). In the evening, a portion of this material is cut off from the nocturnal inversion produced near the ground. Thus, ozone and other smog constituents are removed from the surface scavenging reactions and can persist for longer periods of time. A good demonstration of this process was provided by the flight of DaVinci II on 8-9 June 1976, as shown in Figure 4 (Ripperton *et al.*, 1976).

The DaVinci II experiment involved a surface-operated mobile van and a balloon, which moved along with the wind at an elevation of approximately 750 m. The balloon and van recorded ozone concentration, which increased to approximately 0.13 ppm in the midafternoon when the photochemical generation cycle was active. Overnight, the surface ozone was depleted, but the ozone aloft decreased only slightly. Therefore, many of the reactants and products associated with daytime generation processes remained in the air and were available for further reaction or enhancement of concentration during the next day.

Anderson (1978) proposed a model for nighttime chemistry (see Table 5). The results for an ozone, nitrogen dioxide, and propylene (C_3H_6) system show continued buildup of nitric acid (HNO_3), formaldehyde (CH_2O), and acetaldehyde (CH_3CHO); no sulfur dioxide was added to the reaction scheme. The ozone half-life determined according to this scheme was between 16 and 100 hours, depending upon the initial concentrations in the analyses. These are within the range previously indicated by field measurement for atmospheric ozone. Thus, ozone also would be available the next morning as an added "precursor" for any photochemical smog formation to occur the following day.

The various products of photochemical smog are also involved in the chemistry of sulfuric acid formation. These

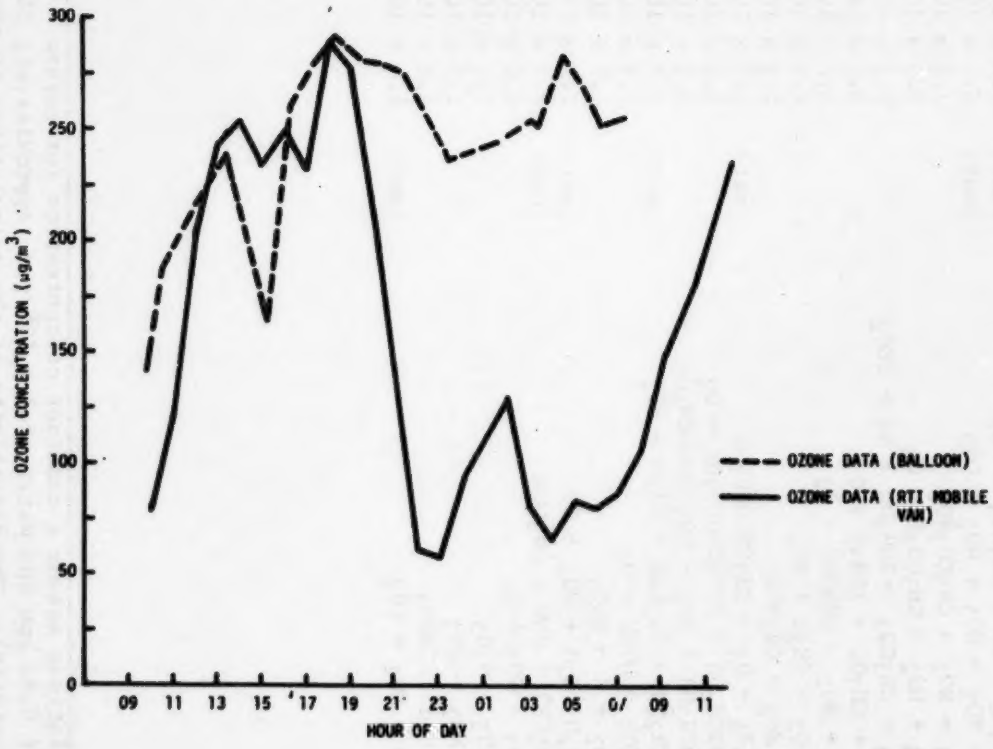


FIGURE 4. Airborne and ground-level ozone concentrations during the flight of DaVinci II on 8-9 June 1976. (From Ripperton et al., 1976)

TABLE 5

Mechanism for Nighttime Chemistry[a]

Reactions		k (x min)
1) $NO + O_3 \rightarrow NO_2 + O_2$		2.1×10^1 ppm^{-1}
2) $O_3 + C_3H_6 \rightarrow HO + HCO_3 + CH_3CHO$	(net)	8.0×10^{-3} ppm^{-1}
3) $O_3 + C_3H_6 \rightarrow HO + CH_3CO_3 + CH_2O$	(net)	9.6×10^{-3} ppm^{-1}
4) $HCO_3 + NO_2 \rightarrow NO_3 + HO_2 + CO_2$	(net)	2.2×10^1 ppm^{-1}
5) $CH_3CO_3 + NO_2 \rightarrow CH_3CO_3NO_2$		2.2×10^1 ppm^{-1}
6) $CH_3CO_3 + HO_2 \rightarrow CH_3CO_3H + O_2$		5.3×10^2 ppm^{-1}
7) $CH_3CO_3 + CH_3CO_3 \rightarrow 2CH_3OO + O_2 + 2CO_2$		3.2×10^2 ppm^{-1}
8) $CH_3OO + CH_3OO \rightarrow 2CH_3O + O_2$		3.2×10^2 ppm^{-1}
9) $CH_3OO + HO_2 \rightarrow CH_3OOH + O_2$		3.2×10^2 ppm^{-1}
10) $CH_3O + O_2 \rightarrow CH_2O + HO_2$		4.8×10^3
11) $CH_3O + NO_2 \rightarrow CH_3ONO_2$		4.9×10^2 ppm^{-1}
12) $OH + C_3H_6 + O_2 \rightarrow CH_3CHOOCH_2OH$	(net)	3.7×10^4 ppm^{-1}
13) $2CH_3CHOOCH_2OH \rightarrow 2CH_3CHOCH_2OH + O_2$		3.2×10^2 ppm^{-1}
14) $CH_3CHOOCH_2OH + HO_2 \rightarrow CH_3CHOHOCH_2OH$		3.2×10^2 ppm^{-1}
15) $CH_3CHOCH_2OH \rightarrow CH_3CHO + CH_2O + HO_2$	(net)	1.5×10^3
16) $HO_2 + HO_2 \rightarrow HOOH + O_2$		5.3×10^3 ppm^{-1}
17) $HO + NO_2 + M \rightarrow HNO_3$		1.1×10^4 ppm^{-1}
18) $HO + CH_2O \rightarrow H_2O + HO_2 + CO$	(net)	2.2×10^4 ppm^{-1}
19) $HO + CH_3CHO \rightarrow H_2O + CH_3CO_3$	(net)	2.2×10^4 ppm^{-1}
20) $NO_2 + O_3 \rightarrow NO_3 + O_2$		4.6×10^{-2} ppm^{-1}
21) $NO_2 + NO_3 \rightarrow N_2O_5$		5.6×10^3 ppm^{-1}
22) $N_2O_5 \rightarrow NO_2 + NO_3$		1.5×10^1
23) $N_2O_5 + H_2O \rightarrow 2HNO_3$		5.0×10^{-6} ppm^{-1}
24) $HO + CO \rightarrow HO_2 + CO_2$	(net)	4.4×10^2 ppm^{-1}

---

[a] All calculations assume a constant concentration for carbon monoxide of 0.85 ppm and water of 1×10^4 ppm (approximately 30% relative humidity). The sensitivity of the calculations to the rate constant for nitric acid formation (reaction 23) was tested using a fast value of 2.5×10^{-3} ppm^{-1} min^{-1} and the value used in this model of 5×10^{-6} ppm^{-1} min^{-1}. No significant difference was observed after 600 minutes. See text for explanation. (From Anderson, 1978)

aerosols are produced from the oxidation of sulfur dioxide, which usually persists for days in the atmosphere since summertime sulfur dioxide is emitted primarily from elevated sources and is transported downwind along with the other stable and free radical species. As shown in Figure 5, sulfur dioxide can remain in the air for several days and travel hundreds of kilometers downwind from its sources.

Furthermore, sulfur dioxide is the only sulfur oxide present in significant concentrations as a vapor. When it is oxidized to sulfur trioxide, which has a high affinity for water vapor, there is a prompt reaction to form sulfuric acid. The molecular-sized droplets of sulfuric acid are hygroscopic and will take an additional water vapor. Moreover, they will almost always be present in such high number concentrations that they will rapidly coagulate, forming fewer but larger droplets. Freshly formed sulfuric acid aerosol in the atmosphere will have median droplet diameters of 0.03 to 0.04 μm, but coagulation shifts the volume median diameter into the relatively stable accumulation mode (0.2 to 0.5 μm) within about 15 minutes. There is very little further change in particle size as the aerosol ages and reacts with ammonia .

OXIDATION OF SULFUR DIOXIDE

Oxidation of sulfur dioxide can take place as a gas-phase reaction, as an aqueous reaction after dissolution in a droplet, or as a reaction on the surface of a solid particle.

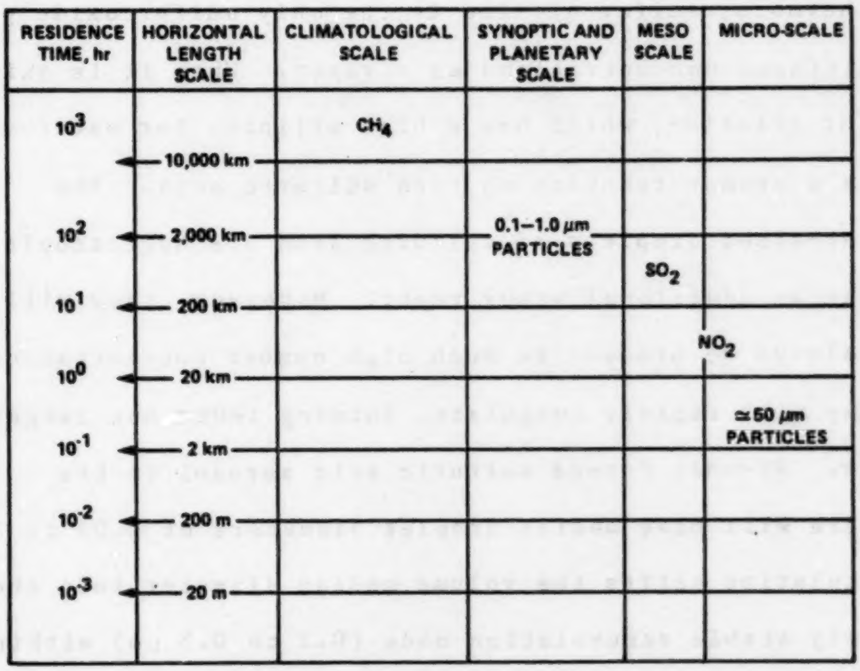

RESIDENCE TIME, hr
HORIZONTAL LENGTH SCALE
CLIMATOLOGICAL SCALE
SYNOPTIC AND PLANETARY SCALE
MESO SCALE
MICRO-SCALE
10^3
10^2
10^1
10^0
10^{-1}
10^{-2}
10^{-3}
10,000 km
2,000 km
200 km
20 km
2 km
200 m
20 m
CH_4
0.1–1.0 μm PARTICLES
SO_2
NO_2
≃50 μm PARTICLES

FIGURE 5. Estimated residence times for select pollutant species and their associated horizontal transport scale. (Air Quality Criteria for Particulate Matter and Sulfur Oxides, U.S. Environmental Protection Agency, 1981)

Gas-Phase Chemical Reactions of Sulfur Dioxide

Homogeneous gas-phase reactions have been most extensively studied and are better understood than any of the others. The U.S.Environmental Protection Agency (1981) recently summarized their pathways and rates. Calvert *et al.* (1978) systematically examined the rate constants and significance of elementary reactions of sulfur dioxide in the troposphere and concluded that many of the reactions were generally unimportant. These included: photodissociation, photoexcitation, reaction with singlet oxygen [(O_2 ('Δg)], reaction with oxygen atom [O(^{3}P)], reaction with ozone, reaction with nitrogen oxides, reaction with *tert*-butylperoxy radical [$(CH_3)_3CO_2$], and reaction with acetylperoxy radical ($RCOO_2$). The only "important" sulfur dioxide reactions in the troposphere were those involving the hydroxyl radical, hydroperoxy radical, and methoxy radical. Table 6 lists the rate constants recommended by Calvert *et al.* (1978) for these three reactions, as well as the different rate constants for the hydroperoxy radical and the methoxy radical reported more recently by Graham *et al.* (1979), Burrows *et al.* (1979), and Sander and Watson (1981). The reasons for the discrepancies among these rate constants are unkown.

Although the dark reaction of sulfur dioxide plus ozone is too slow to be important in the troposphere, the addition of alkenes greatly enhances the oxidation rate. Calvert *et al.* (1978) reviewed and reevaluated the experimental work of Cox

TABLE 6

Rate Constants for Hydroxyl (HO), Hydroperoxy (HO_2), and Methoxy (CH_3O_2) Radicals

Reaction	Second-Order Rate Constant ($cm^3 mole^{-1} s^{-1}$)	Reference
$HO + SO_2 \rightarrow HOSO_2 \rightarrow H_2SO_4$	$(1.1 \pm 0.3) \times 10^{-12}$	Calvert *et al*., 1978
$HO_2 + SO_2 \rightarrow HO + SO_3 \rightarrow H_2SO_4$	$> (8.7 \pm 1/3) \times 10^{-16}$	Calvert *et al*., 1978
	$< 1 \times 10^{-18}$	Graham *et al*., 1979
	$\leq 2 \times 10^{-17}$	Burrows *et al*., 1979
$CH_3O_2 + SO_2 \rightarrow CH_3O + SO_3 \rightarrow H_2SO_4$	$(5.3 \pm 2.5) \times 10^{-15}$	Calvert *et al*., 1978
	5×10^{-17}	Sander and Watson, 1981

and Penkett (1971a, 1971b) and McNelis *et al*. (1975). The reaction system is highly complex: For total alkenes at 0.10 ppm, ozone at 0.15 ppm, and sulfur dioxide at 0.05 ppm., Calvert *et al*. (1978) estimated that the disappearance rate of sulfur dioxide is 0.23 and 0.12% hour^{-1} at 50% and 100% relative humidity (25°C). Niki *et al*. (1977) and Su *et al*. (1980) studied the reaction mechanism for the ozone plus alkene plus sulfur dioxide system, but it is still not well established.

In summary, the status of our knowledge of the gas-phase tropospheric sulfur dioxide oxidation reactions consists of the following:

1. Three reactions are potentially important:
    a. Hydroxyl radical--The rate constant appears to be well established.
    b. Hydroperoxy radical--The rate constant is not well established.
    c. Methoxy radical--The rate constant is not well established.
2. The sulfur dioxide plus ozone plus alkenes reaction may be an important dark reaction.

Typical rates of sulfur dioxide oxidation were of the order of 1.5% per hour and 40% per hour for clean and polluted atmospheres, respectively, during July at midnorthern latitudes; the major difference in rates was a result of higher concentration levels of free radicals in the hydrocarbon-rich, photochemical reactive atmospheres. Altshuller (1979) predicted the rates of homogeneous oxidation of sulfur dioxide to sulfate in the clean troposphere using concentration predictions of the pertinent free radicals from a two-dimensional global model by Fishman and Crutzen (1978). Figure 6 presents a sample result from this study showing the latitudinal and seasonal dependence of the rate of sulfur dioxide oxidation; the variability in rate was predominantly due to availability of ultraviolet solar intensity, which drives the free-radical production process.

The solar radiation dependence of the sulfur dioxide conversion rate has also been observed in field measurements within power plant plumes (Husar et al., 1978), but these results should be viewed cautiously in light of the complicating factors introduced by the dispersion and local chemistry of the primary source emissions. Figure 7, which shows the diurnal variation of aerosol formation in this plume, reveals that the aerosol formation during the first week after sulfur dioxide emission depends upon the time of day it was emitted.

Experimental determinations of the reaction rate constants of sulfur dioxide with hydroperoxy radical by Graham et al. (1979) and by Burrows et al. (1979) and of sulfur dioxide with methoxy radical by Sander and Watson (1981) show that hydroperoxy and methoxy radicals must be considered as questionable reactants contributing to the oxidation of sulfur

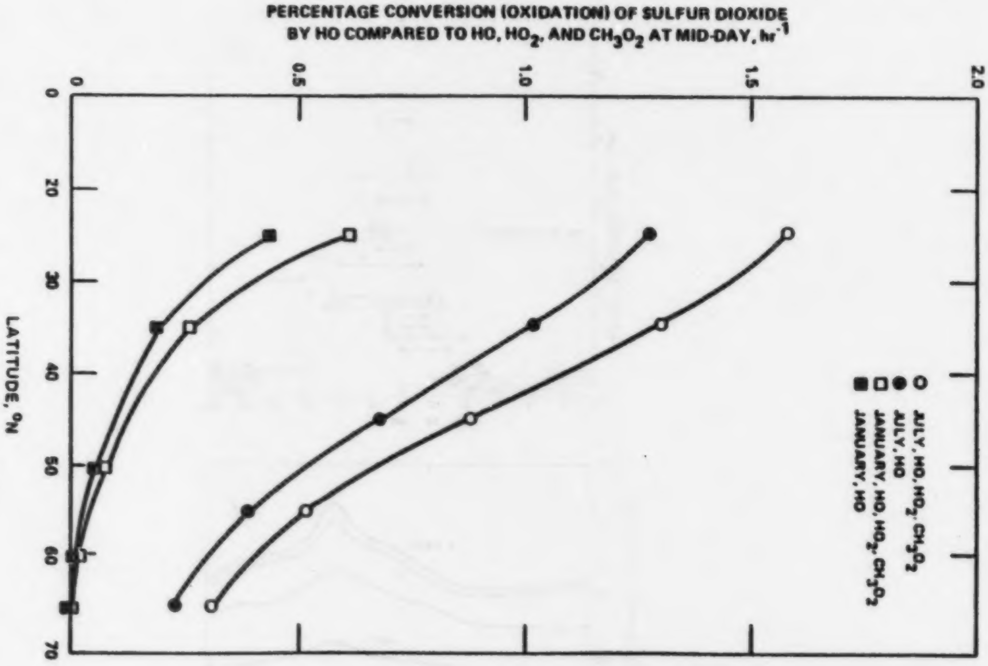


FIGURE 6. Percentage conversion at midday of sulfur dioxide by the hydroxyl radical (HO) and by HO, the hydroperoxy radical (HO_2), and the methoxy radical (CH_3O_2) as function of °N latitude in summer and winter. (From Air Quality Criteria for Particulate Matter and Sulfur Oxides, U.S. Environmental Protection Agency, 1981)

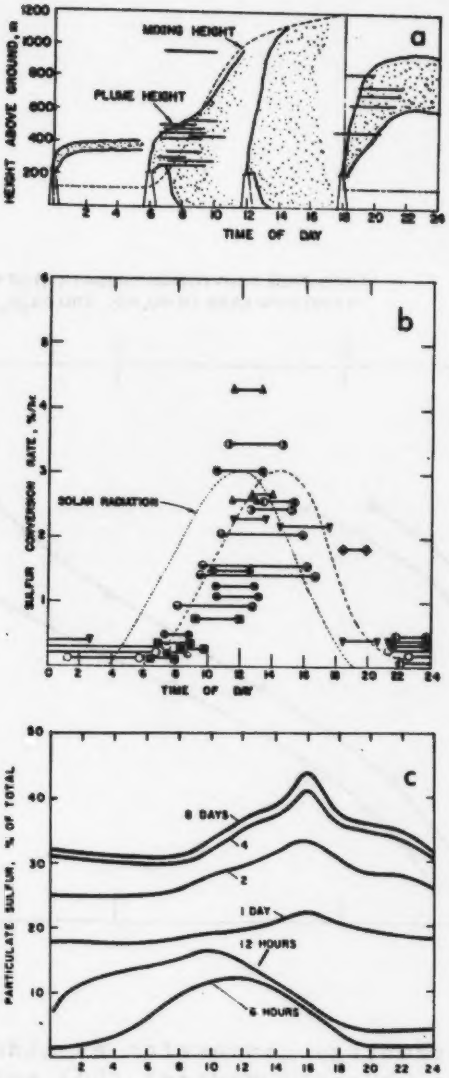


FIGURE 7. Diurnal pattern of (a) plume dispersion, (b) conversion rate, and (c) fraction of sulfur converted to aerosol in a midwestern power plant for different emission times. (From Sulfur Oxides, National Academy of Sciences, 1978)

trioxide in the atmosphere. This results in maximum established sulfur dioxide oxidation rates of the order of 1.5% per hour for both clean and polluted atmospheres during July at midnorthern latitudes, which is a factor two and one-half times lower than previous theoretical estimates for polluted atmospheres. The revised rate is equivalent to a diurnally averaged rate of the order of 0.4% per hour. Field measurements on the rates of sulfur dioxide oxidation indicate that maximum sulfur dixoide oxidation rates of the order of 10% per hour are typical of many atmospheric pollution scenarios.

Our present knowledge of homogeneous sulfur dioxide gas-phase reactions does not sufficiently account for the rates observed. Smog chamber studies have demonstrated that some species other than hydroxyl radical oxidize sulfur dioxide (Kuhlman *et al*., 1978; McNelis *et al*., 1975). Alternate homogeneous-gas-reaction oxidation pathways are being studied (Su *et al*., 1980), but certainly the role of heterogeneous and liquid-phase sulfur dioxide oxidation pathways must not be overlooked in attempts to resolve this discrepancy.

Liquid-Phase Reactions of Sulfur Dioxide

Sulfur dioxide dissolves in water (H_2O) to form $SO_2 \cdot H_2O$, HSO_3^-, and sulfite (SO_3^{2-}). Although the formation of sulfurous acid (H_2SO_3) is often postulated instead if $SO_2 \cdot H_2O$, it has not been observed (Lyons and Nickless, 1968). The formation of these species in water occurs through the equilibrium reactions given in Table 7.

TABLE 7

Dilute Sulfur Dioxide-Water ($SO_2 + H_2O$) System

Reaction	Constant (25° C)
$SO_{2(g)} \rightleftarrows SO_2 \cdot H_2O$	H = 0.0332[a]
$SO_2 \cdot H_2O \rightleftarrows H^+ + HSO_3^-$	$K_{A1} = 1.39 \times 10^{-2}$[b] $pK_{A1} = 1.86$
$HSO_3^- \rightleftarrows H^+ + SO_3^{2-}$	$K_{A2} = 4 \times 10^{-8}$[c] $pK_{A2} = 7.40$

---

[a] H = Henry's law constant (dimensionless), which is $SO_{2(g)}$(gaseous) molar concentration divided by $SO_2 \cdot H_2O$ molar concentration (Hales and Sutter, 1973).

[b] K_{A1} = dissociation constant in moles per liter (Huss and Eckert, 1977).

[c] K_{A2} = dissociation constant in moles per liter (Salomaa et al., 1969).

Eigen et al. (1961) measured the forward (k_{+1}) and reverse (k_{-1}) rate constants at 20°C for the following reaction:

$$SO_2 \cdot H_2O \rightleftarrows H^+ + HSO_3^-$$

They found that:

$$k_{+1} = 3.4 \times 10^6 s^{-1} \text{ and } k_{-1} = 2 \times 10^8 M^{-1} s^{-1}.$$

These measurements demonstrate that the $SO_2 \cdot H_2O$ - HSO_3 reaction will achieve equilibrium with 1 microsecond perturbation.

The rate constants k_{+2} and k_{-2} are not known for the following reaction:

$$HSO_3^- \rightleftarrows H^+ + SO_3^{2-}$$

The value of the protonation rate (k_{-2}) is probably less than the theoretical diffusion limit ($\sim 5 \times 10^{10} M^{-1} s^{-1}$), but greater than k^{-1}. The expected range of k_{+2} is therefore $0.008\text{-}2 \times 10^3 s^{-1}$, which means that SO_3^{2-} will achieve equilibrium concentration within 0.5 to 125 milliseconds of perturbation. Thus, the equilibrium distribution of the $SO_2 \cdot H_2O$, HSO_3^-, and SO_3^{2-} is expected to achieve chemical equilibrium with a relaxation time of 0.5 to 125 milliseconds. This time is too short to affect sulfate formation rates in particles, mists, and rain; that is, equilibrium conditions are continuously satisfied in these liquid systems. An important feature of the $SO_2 \cdot H_2O$ - HSO_3^- - SO_3^{2-} system is the influence of hydrogen ion (H^+) in governing the distribution of these species (see Figure 8).

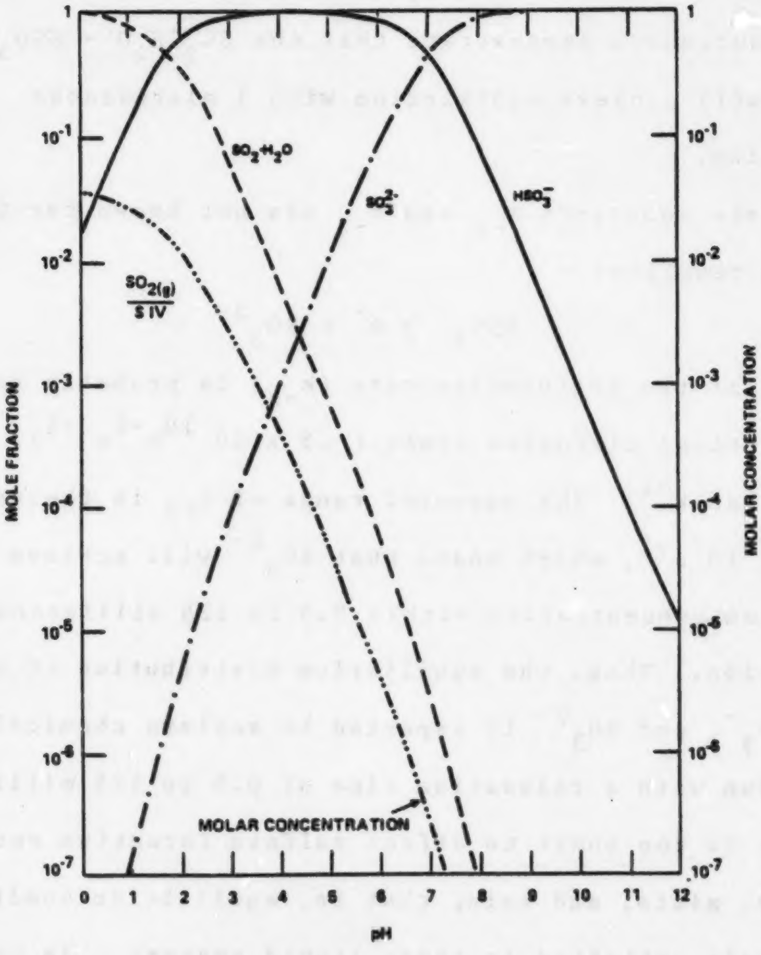


FIGURE 8. The distribution of species for the $SO_2 \cdot H_2O$ - HSO_3^--SO_3^{2-} systems as a function of pH. (From Air Quality Criteria for Particulate Matter and Sulfur Oxides, U.S. Environmental Protection Agency, 1981)

Sulfite ions form stable complexes with many metal ions, especially those in Periodic Group VIII (Lyons and Nickless, 1968). The formation of the stable complex dichlorosulfitomercuate ion is the basis of the West-Gaeke method for determining sulfur dioxide in the air.

A knowledge of the reactions of the aqueous $SO_2 \cdot H_2O$ - HSO_3^- and SO_3^{2-} system is important to understanding the process of sulfuric acid formation in tropospheric particles, mists, fogs, and rain. This section reviews the oxidation reaction of dissolved sulfur dioxide species, including the auto-oxidation, metal-ion-catalyzed oxidation, carbon-catalyzed oxidation, and reactions with the dissolved oxidants, nitrogen dioxide, ozone, and hydrogen peroxide.

The state of knowledge of aqueous oxidation rates of dissolved sulfur dioxide, the dissociation product HSO_3^-, and sulfite is inadequate for simple systems and is extremely poor (or nonexistent) for complex systems that include dissolved nitrogen and carbon compounds. Unfortunately, most of the studies are not definitive because the investigators (a) did not provide sufficient descriptions of experimental procedures, especially the purification of the water and reagents; (b) did not select a proper reactor design; and (c) worked at concentration levels that were orders of magnitude greater than were possible for ambient aqueous systems. Trace quantities (at the part-per-billion level) of catalytic metalions are capable of enhancing the reaction velocities by orders of magnitude over the auto-oxidation rate, while similar trace quantities of organics inhibit the rate.

The characteristics of the chemical reactor govern the range of the half-life that can be investigated and may influence the observed rate of oxidation. Two-phase air-water reactors (e.g., bubblers and supported droplets) may have reaction characteristics that depend upon the mass-transfer rate of the reactants through the air-water interface and the mixing rates within the gas and water phases (Carberry, 1976; Freiberg and Schwartz, in press). Supported droplets may suffer from an additional problem: Radical chains are efficiently terminated at liquid-solid interfaces, thereby reducing the observed rate. Therefore, supported droplet measurements are not defensible unless the oxidation is not a free-radical mechanism. Several notable reviews of the oxidation of dissolved sulfur dioxide and its hydration products in simple systems have been published by Schroeter (1963) and Hegg and Hobbs (1978).

The U.S. Environmental Protection Agency review (1981) made the following conclusions:

1. The auto-oxidation (uncatalyzed) reaction is very slow compared to other reactions and:
    a. the rate is extremely sensitive to the presence of catalysts and inhibitors;
    b. the rate is first-order in sulfate;

c. no reaction mechanism has been satisfactorily demonstrated to account completely for the observations of the dependence of the rate on $[H^+]^{0.5}$.

2. Mn(II) manganese and Fe(III) iron are significant catalysts for the oxidation. The kinetic rate expression is in doubt for the Mn(II) manganese reaction, but several independent investigators concur on that for Fe(III) iron.

3. There is relatively little known about other aspects of the homogeneous metal-ion catalysis systems. Those currently inadequately characterized include Cu(II) copper, V(V) and V(IV) vanadium, Ni(II) nickel, Zn(II) zinc, and Pb(II) lead. Also, there are no quantitative studies of metal-ion/metal-ion synergism, and the ability of atmospheric organic compounds to inhibit the catalysis is unknown. All studies have been performed in the absence of bicarbonate (HCO_3^-); however, the following reactions may be important:

$$^{\bullet}SO_4^- + HCO_3^- \rightarrow {}^{\bullet}HCO_3 + SO_4^{2-}$$

$$^{\bullet}OH + HCO_3^- \leftarrow {}^{\bullet}HCO_3 + OH^-$$

If such reactions occur, they would prevent the establishment of the radical chain oxidation since biocarbonate is not a powerful oxidizer. Finally, the rate expression for catalytic oxidation to form sulfuric acid is not well-established.

4. Elemental carbon (soot) with a water film is a potentially effective oxidation catalyst.

5. The status of knowledge of the formation of sulfuric acid

by dissolved oxidants consists of the following:

a. The oxidation rates are known for nitric oxide and nitrite (NO_3^-) and are too low to be important.

b. The oxidation rate is known for nitrogen dioxide, but the tropospheric concentration of nitrous acid is probably too low for this reaction to be important.

c. The oxidation rate is known for ozone, but it is usually unimportant.

d. The oxidation rate for hydrogen peroxide (H_2O_2) is known and appears to be potentially a highly effective reaction for formation of sulfuric acid in the troposphere. This rate could possibly be enhanced by metal ions, but no studies have been reported.

e. Organic oxidizers may be important, but no studies have been reported.

The Influence of Ammonia

Ammonia may influence the formation rate of sulfuric acid in aqueous particles, mist, fog, and rain. When Hegg and Hobbs (1978) reviewed the studies of the influence of ammonia, they called attention to a misunderstanding in the literature: Ammonia is commonly reported incorrectly to be a "positive catalyst" for the oxidation of dissolved sulfur dioxide. In the strict sense of the definition of "catalyst," the term cannot be applied to the role of ammonia. The observed enhancement by ammonia of the oxidation rates of the auto-oxidation, metal-ion oxidation, and ozone oxidation is due to its action in raising and maintaining a high pH. The following process occurs to raise and maintain a higher pH through the conversion of ammonia to ammonium ion (NH_4^+): First, ambient gaseous ammonia dissolves in the water: $NH_{3(g)} \rightleftarrows NH_{3(aq)}$. Then, the dissolved ammonia reacts with hydrogen ion, which raises the pH: $NH_{3(aq)} + H^+ \rightleftarrows NH_4^+$. Therefore, the ambient pathways of auto-oxidation, Mn(II) manganese- and Fe(III) iron- catalyzed oxidation, and ozone oxidation would have their rates enhanced by absorption of ammonia. However, the ambient pathways of hydrogen peroxide and nitrous acid would have their rates retarded by absorption of ammonia. The rate for soot would not be influenced.

There are other important roles for ammonia. Reinders and Vles (1925) observed qualitatively that ammonia complexed Cu(II) copper and rendered it noncatalytic. At high pH (> 9) such that aqueous ammonia is the dominant form, ozone and free radicals may oxidize ammonia (Hoigne and Bader, 1978). Thus, the role of ammonia is explained in terms of influence on the pH of the water system; it is not a catalyst.

Surface Chemical Reactions of Sulfur Dioxide

Industrial emissions of solid particles (e.g., flyash) and fugitive dust (e.g., windblown soil and minerals) provide a solid surface that may chemisorb sulfur dioxide and yield sulfate ions. This section will cover investigations of the sulfur dioxide oxidation on the surfaces of metal oxides, flyash, charcoal, and soot. Although reaction kinetics have not been identified, two general types of processes have: a capacity-limited reaction for sulfur dioxide removal and a catalytic sulfur dioxide-oxidation process. The initial contact of sulfur dioxide with the solid produces a rapid loss of sulfur dioxide from the gas phase; the reaction rate decreases with time. For the capacity-limited reaction, the rate slowly approaches zero for the catalytic process; the rate levels off for a time and then approaches zero. The latter phenomenon is attributed to a pH decrease caused by sulfuric acid formation.

Current knowledge of surface reactions can be summarized as follows:

1. The reactions are capacity-limited. Those that involve catalysis in liquid films can be extended by the absorption of ammonia.

2. The initial rates may be large, but quickly approach zero.

3. Except for the carbon (soot) reaction, solid surface reactions are not effective pathways for sulfuric acid formation in the troposphere.

Estimates of Sulfur Dioxide Oxidation

Tables 8 and 9 present a comparison of the rates of gas-phase and aqueous-phase oxidation with the rates of sulfur

TABLE 8

Estimates of Gas-Phase Sulfur Dioxide Oxidation Rates in Well-Mixed Troposphere

Reaction	Rate (% $hour^{-1}$)[a]
Hydroxyl radical (HO)	0.3 - 1.3
Hydroperoxy radical (HO_2)	0.4 - 2.0
Methoxy radical (CH_3O_2)	0.3 - 1.5

[a] Typical range for daytime at northern midlatitudes during the summer; the reaction rates for hydroperoxy and methoxy radicals are not well established; see text for details.

TABLE 9

Estimates of Aqueous-Phase Sulfur Dioxide Oxidation Rates in Well-Mixed Troposphere

Reaction[a]	Rate		
	pH 1	pH 3	pH 5
Mn(II)manganese catalysis[b]	1×10^{-1}	$1 \times 10^{+1}$	$1 \times 10^{+3}$
Fe(III) iron catalysis[c]	5×10^{-5}	5×10^{-1}	$5 \times 10^{+3}$
Carbon (soot) catalysis[d]	$3 \times 10^{+1}$	$3 \times 10^{+1}$	$3 \times 10^{+1}$
Ozone (O_3)[e]			
at 40 parts per billion	2×10^{-8}	2×10^{-6}	2×10^{-4}
at 120 parts per billion	6×10^{-8}	6×10^{-6}	6×10^{-4}
Hydrogen peroxide (H_2O_2)[f]			
at 1 part per billion	2×10^{-2}	3×10^{-2}	3×10^{-2}
at 10 parts per billion	2×10^{-1}	2×10^{-1}	3×10^{-1}

---

[a] In all cases except carbon catalysis, it is assumed that liquid water volume of the aerosol is $50 \times 10^{-12} m^3/m^3$ and that gaseous sulfur dioxide is 10 ppb (or 27 $\mu g/m^3$).

[b] Assumed that Mn(II) manganese mass concentration is 20 ng/m^3, and that Mn(II) manganese is uniformly dissolved in the liquid water of the aerosol: Mn(II) = 8.9×10^{-3} M. Rate calculation used the expression of Neytezell-de Wilde and Taverner (1958). This reaction rate is not well-established; see text for details.

[c] Assumed that Fe(III) iron mass concentration is 2 $\mu g/m^3$ and that Fe(III) iron is uniformly dissolved in the liquid water of the aerosol: Fe(III) = 0.9 M. Rate calculation used the expression of Neytzell-de Wilde and Taverner (1958).

[d] Assumed that carbon mass concentration is 10 $\mu g/m^3$ and behaves as the soots studied by Chang *et al*. (1979), whose expression was used for this calculation.

[e] Rate calculation was based on Equation 2-35 (U.S. Environmental Protection Agency, 1981).

[f] Rate calculation was based on Equation 2-39 (U.S. Environmental Protection Agency, 1981).

dioxide oxidation for an assumed set of conditions. These calculations ignore the nonhomogeneous nature of the troposphere and assume that all of the reactants are well mixed. For this comparison, it was assumed that the sulfur dioxide concentration is 10 parts per billion (ppb) for all of the reactions, and that the liquid water content of the aerosol is 50×10^{-12} m^3/m^3.

Several of the assumptions made do not have any basis, namely:

1. The ambient mass concentration of 20 nanograms (ng)/m^3 for manganese is reasonable, but it is not known if the predominant form is Mn(II) manganese, and it is unlikely that manganese is uniformly distributed and dissolved.

2. Likewise, the ambient concentration of 2 $\mu g/m^3$ for iron is reasonable, but it is not known if Fe(III) iron is the predominate form, and it is unlikely that iron is uniformly distributed and dissolved.

3. There is no basis to assume that the rate equation observed for laboratory-generated carbon (soot) applies to atmospheric carbon.

4. The reaction rates for hydroperoxy and methoxy radicals recommended by Calvert et al. (1978) are not well-established.

It is very likely that the rates calculated for Mn(II) manganese catalysis, Fe(III) iron catalysis, and carbon (soot) catalysis are gross overestimates. Furthermore, hydroperoxy and methoxy radical rates may be too high. Uncritically accepting all of the rates, at a pH of 3 and a level of hydrogen peroxide at 10 ppb, the sulfur dioxide conversion rate would exceed 40% hour^{-1}. However, if only the well-established rates are considered, the sulfur dioxide conversion rate becomes $\sim$1.1% hour^{-1}.

The following facts summarize the status of our knowledge of sulfur dioxide-oxidation pathways:

1. The gas-phase reaction rate of hydroxyl radical and the aqueous-phase reaction of hydrogen peroxide are well established, but are expected to account only at 1.1% hour^{-1} (under the conditions given in Tables 8 and 9).

2. The Mn(II) manganese-, Fe(III) iron-, and carbon (soot)-catalyzed reactions have sufficient rates to dominate sulfur dioxide oxidation in the troposphere, but we do not have confidence that our assumptions are reasonable.

MOLECULAR FORMS OF SULFUR IN TROPOSPHERIC AEROSOLS

Oxidation State +6

Atmospheric sulfuric acid particles (and their neutralization products with ammonia) are produced both at the source and in the atmosphere by the oxidation of sulfur dioxide and are found preferentially in the accumulation mode, i.e., in particles, between 0.1 and 1.0 μm diameter. They are all hygroscopic and will take up water vapor and grow in droplet size at high relative humidities (see Figure 9).

The combination of usual tropospheric temperatures (from -20°C

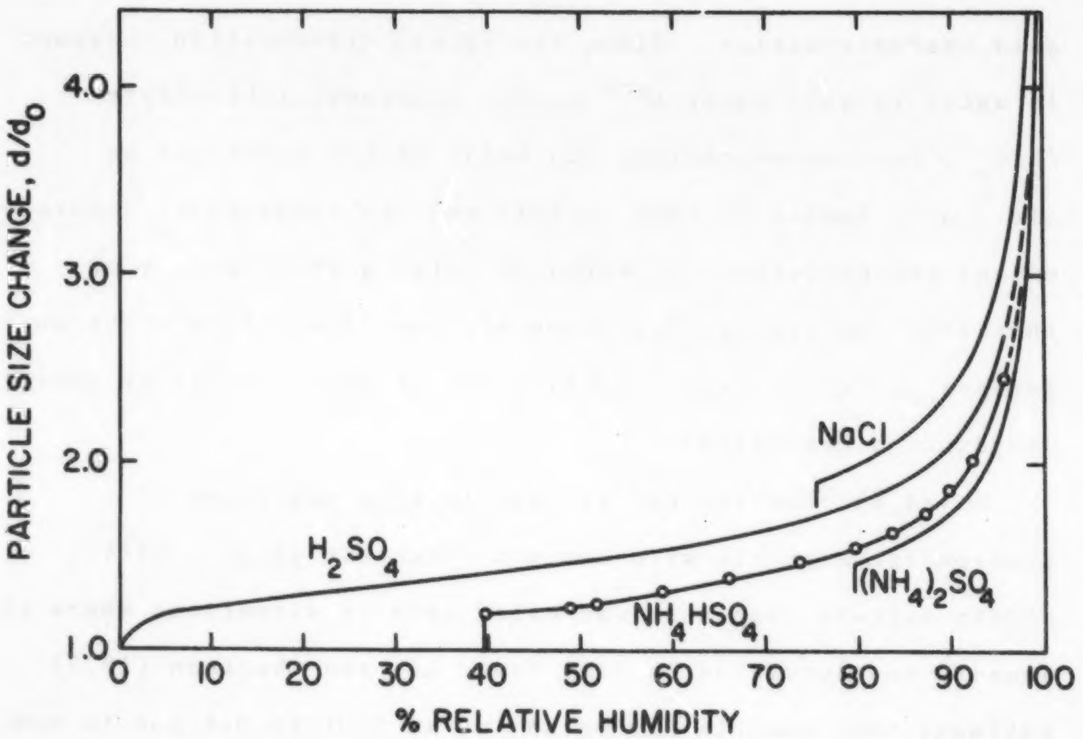


FIGURE 9. Theoretical growth curves for solution droplets of sulfuric acid and other inorganic salts of interest at 25°C. Key: H_2SO_4 = sulfuric acid, NaCl = sodium chloride, NH_4HSO_4 = ammonium acid sulfate, $(NH_4)_2SO_4$ = ammonium sulfate. (From Sulfur Oxides, National Academy of Sciences, 1978)

to +40°C) and humidities (20% to 100% relative humidity) along with its hygroscopic properties dictate that sulfuric acid is highly hydrated and usually liquid (Tang, 1976). As a consequence of the large concentration ratios of water to sulfuric acid above about 30% relative humidity, sulfuric acid aerosol becomes highly dissociated and thus exhibits strong acid characteristics. Since the second dissociation constant in water is only about 10^{-2} molar, substantial bisulfate (HSO_4^-) ion concentrations can exist in the particles in air. As a result of this acidity and the fundamental nature of modest concentrations of sulfuric acid, a wide variety of inorganic and organic reactions are possible within or on such aerosol particles (e.g., dissolution of metal oxides or organic dehydration reactions).

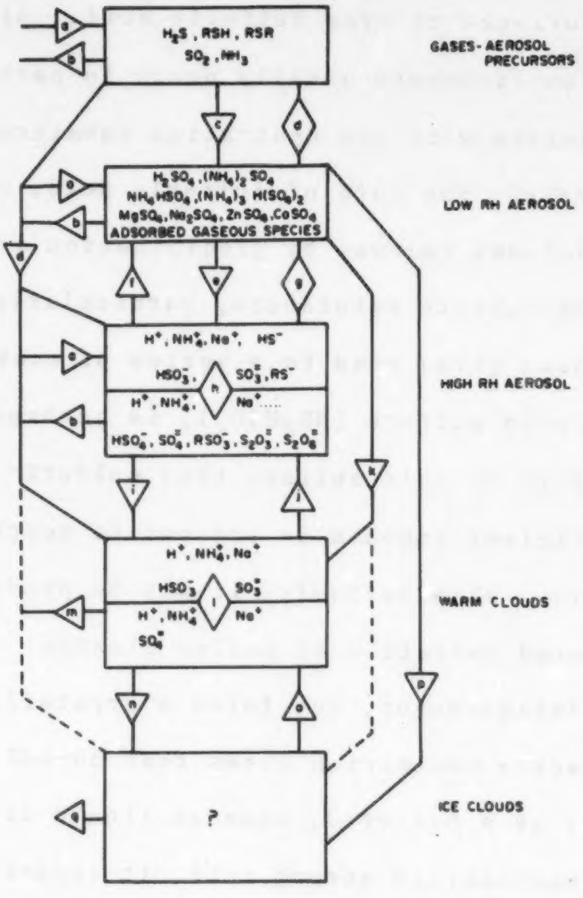
Based on observations of fast _in situ_ reactions of atmospheric aerosols with ammonia (Charlson _et al_., 1974), acidic sulfate compounds can exist only in situations where the ammonia concentration is very low. Lau and Charlson (1977) estimate that ammonia may be as low as 0.01 to 0.1 ppb in some regions, notably those areas with acidic soils, as suggested by Junge (1963).

Aerosols of sulfuric acid may also exist briefly near the source as a direct product of oxidation (e.g., in chimney plumes or automobile exhaust, where ammonia is almost completely scavenged by an excess of free acid). There may be other alkaline gases of concern (such as amines), which would limit the occurrence of free sulfuric acid. Alkaline dusts such as calcium carbonate usually occur in particle sizes too large to coagulate with and neutralize submicrometer sulfuric acid effectively. The fate of sulfuric acid, as indicated in Figure 10, includes removal by precipitation or the reaction with other atmospheric substances, particularly ammonia. The reaction pathway gives rise to a series of compounds.

Ammonium acid sulfate (NH_4HSO_4), is perhaps an even more common form of acid sulfate than sulfuric acid. It occurs whenever sufficient ammonia is present to neutralize sulfuric acid partially. Alternatively, it may be produced directly by ammonia-enhanced oxidation of sulfur dioxide. Unlike sulfuric acid, it is deliquescent, but forms a crystalline salt only at very low relative humidities (less than 30-40%). Thus, it will usually exist as a hydrated, aqueous liquid droplet aerosol. As the half-neutralized strong acid, it contributes to low pH in rain and aerosol particles.

Letovicite [$(NH_4)_3H(SO_4)_2$] occurs less frequently than the more acidic sulfate compounds or the completely neutralized form of ammonium sulfate [$(NH_4)_2SO_4$]. According to Tang and Munkelwitz (1977), letovicite is deliquescent at about 68% relativehumidity, and thus will often exist as solution droplets, which was also the case for sulfuric acid or ammonium sulfate.

Ammonium sulfate, which is the fully neutralized ammonium salt of sulfuric acid, is deliquescent at 80% relative


Chemical properties of tropospheric sulfur aerosols

H_2S, RSH, RSR
SO_2, NH_3
GASES-AEROSOL PRECURSORS
H_2SO_4, $(NH_4)_2SO_4$
NH_4HSO_4, $(NH_4)_3H(SO_4)_2$
$MgSO_4$, Na_2SO_4, $ZnSO_4$, $CaSO_4$
ADSORBED GASEOUS SPECIES
LOW RH AEROSOL
H^+, NH_4^+, Na^+, HS^-
HSO_3^-, $SO_3^=$, RS^-
H^+, NH_4^+, Na^+.....
HSO_4^-, $SO_4^=$, RSO_3^-, $S_2O_3^=$, $S_2O_6^=$
HIGH RH AEROSOL
H^+, NH_4^+, Na^+
HSO_3^- $SO_3^=$
H^+, NH_4^+ Na^+
$SO_4^=$
WARM CLOUDS
?
ICE CLOUDS
a
b
c
d
e
f
g
h
i
j
k
l
m
n
o
p
q

FIGURE 10. The tropospheric sulfur cycle Key: □ recognizable entities in the atmosphere; △ processess having single direction of material flow; ◇ reversible processes; (a) sources; (b) sinks; (c) gas-to-particle conversions; (d) sorption; (e) deliquescence; (f) efflorescence; (g) Raoult's equilibrium; (h) reaction in concentrated solution droplet; (i) nucleation and condensation of water; (j) evaporation; (k) capture of aerosol by cloud drops; (l) reaction in dilute solution; (m) rain; (n) freezing of supercooled drop by ice nucleus; (o) melting; (p) direct sublimation of ice on ice nucleus; (q) precipitation. R = organic radical; H_2S = hydrogen sulfide; SO_2 = sulfur dioxide; NH_3 = ammonia; H_2SO_4 = sulfuric acid; $(NH_4)_2SO_4$ = ammonium sulfate; NH_4HSO_4 = ammonium acid sulfate; $(NH_4)_3H(SO_4)_2$ = letovicite; $MgSO_4$ = magnesium sulfate; Na_2SO_4 = sodium sulfate; $ZnSO_4$ = zinc sulfate; $CaSO_4$ = calcium sulfate. (From Air Quality Criteria for Particulate Matter and Sulfur Oxides, U.S. Environmental Protection Agency, 1981)

humidity. It is only weakly acidic in aqueous solutions due to the hydrolysis of the ammonium ion. Because it is an almost unreactive material, it is often the last and most stable compound formed prior to removal from the atmosphere. As a secondary aerosol, ammonium sulfate is found in well-aged air masses, notably those in which ammonia is abundant.

Atmospheric measurements supporting the existence of sulfuric acid and its neutralization products with ammonia include the following:

- the _in situ_ reaction of submicrometer particles with ammonia (gas) (Charlson _et al_., 1974); this method does not differentiate between ammonium acid sulfate and sulfuric acid;
- infrared spectra of collected samples (Cunningham and Johnson, 1976);
- Raman spectra of collected samples (Rosen and Novakov, 1977);
- titration of collected samples (Brossett _et al_., 1974);
- the volatility of collected samples (Roberts and Friedlander, 1975);
- the _in situ_ volatilization and measurement of sulfuric acid and ammonium salts (Cobourn _et al_., 1978; Morandi _et al_., 1981); the volatility methods do not differentiate between ammonium sulfate and ammonium acid sulfate;

- laboratory simulations in which sulfate compounds are produced photochemically (Kuhlman et al., 1978);
- chemical model calculations in which sulfuric acid is a logical product of oxidation of sulfur dioxide; and
- precipitation composition studies in which sulfuric acid is the dominant free acid.

Oxidation State +4

Novakov et al. (1972), using x-ray photoelectron spectroscopy, identified S(IV) sulfur in fine particles collected on filters in Pasadena, California. He suggested that the S(IV) sulfur is chemisorbed and subsequently oxidized on the particle surface. S(IV) sulfur concentrations were estimated to be as high as 6 $\mu g/m^3$, although enrichment of S(IV) sulfur at the particle surface probably makes this an overestimate of the total mass loading of S(IV) sulfur. Rosen and Novakov (1977) have recently identified graphitic carbon as the likely absorption surface.

Certain metal cations (e.g., Hg^{2+} mercury, Fe^{3+} iron, Cu^{2+} copper) will form metal sulfite or metal hydroxy sulfite complexes in the presence of dissolved sulfur dioxide. Depending on the molar ratio of metal to sulfur, the sulfur will be tightly bound to the metal, will be stable to air oxidation, and will not outgas from solution upon droplet evaporation (Hansen et al., 1976).

Eatough et al. (1977), using thermometric titration with potassium dichromate (VI), have identified S(IV) sulfur in aerosol collected on filters near a copper smelter and in trace amounts in a sample from New York City. They suggest that these are transition metal complexes based on Mossbauer and photoelectron spectra of samples taken in the work environment of a copper smelter and on correlation studies of S(IV) sulfur concentrations with Fe^{3+} iron and Cu^{2+} copper concentrations in ambient samples. They found that more than 80% of the S(IV) sulfur is in the coarse mode, accounting for about 1-2% of the total aerosol mass. Assuming a submicrometer aerosol mass concentration of 50 $\mu g/m^3$, this means that about 0.1 $\mu g/m^3$ metal sulfites would be present in ambient air, which is roughly 1-10% of the mass concentration found by Novakov *et al*. (1972). At times, the metal ion complexes appear to represent an important fraction of the total S(IV) sulfur in the submicrometer, subsaturated aerosol.

ATMOSPHERIC REACTIONS OF ACIDIC DROPLETS

The original composition of the water-soluble part of the particles can be regarded, at least at sufficiently high relative humidity, as a solution of the nonvolatile components sulfuric acid and MOH, where M represents all cations from basic oxides (in water). Here, the acid will be dominant in the fine particles and the alkali in the coarse mode. In the atmosphere, this liquid phase is in contact with a gas phase (air) that contains, among other things, the components of interest here, i.e., ammonia, nitric acid, and hydrochloric acid (HCl). In a state of equilibrium, the liquid phase will thus also contain the ions H^+, NH_4^+, NO_3^-, and Cl^-.

Thus the system consists of two phases containing six components; therefore it has six degrees of freedom. This means that, by selecting each concentration (C), such as $C_{SO_4}{}^{2-}$ and $C_M{}^+$, and each partial pressure (P)--P_{NH_3}, P_{HNO_3}, and P_{HCl} as independent variables and giving them definite values (the variable water), we can, for equilibrium conditions, calculate the concentrations in the liquid phase of $C_H{}^+$, $C_{NH_4}{}^+$, $C_{NO_3}{}^-$, and C_{HCl}. In such a case, the following equation is valid:

$$2C_{SO_4}{}^{2-} - C_M{}^+ = C_H{}^+ + C_{NH_4}{}^+ - C_{NO_3}{}^- - C_{Cl}{}^-$$

When $C_H{}^+/C_{NH_4}{}^+ = k_1 \cdot (P_{NH_3})^{-1} = q$, $C_H{}^+ \cdot CNO_3{}^- = k_2 \cdot P_{HNO_3} = r$, and

$C_H{}^+ \cdot C_{Cl}{}^- = k_3 \cdot P_{HCl} = s$, then:

$$C_H{}^+ = \frac{2C_{SO_4}{}^{2-} - C_M{}^+}{2\left(1 + \frac{1}{q}\right)} + \left[\frac{(2C_{SO_4}{}^{2-} - C_M{}^+)^2}{4\left(1 + \frac{1}{q}\right)^2} + \frac{r + s}{1 + \frac{1}{q}}\right]^{1/2}$$

where $C_{NH_4}{}^+ = \frac{C_H{}^+}{q}$, $C_{NO_3}{}^- = \frac{r}{C_H{}^+}$, and $C_{Cl}{}^- = \frac{s}{C_H{}^+}$.

The application of the first can be illustrated by assuming that there are two particle populations, corresponding to fine and coarse particles, respectively, in equilibrium with a gas phase. The independent variables, chosen on the basis of previous experience, are shown below:

Particles (liquid phase mol/liter)

	Fine	Coarse
$2C_{SO_4^{2-}}$	4.00	0.30
C_{M^+}	0.00	4.00

Gas Phase

$q = 0.2;\ r = 0.2;\ s = 0.1$

The equation then yields the following composition for the water-soluble part of the particles (mol/liter):

	Fine	Coarse
C_{H^+}	0.73	0.07
$C_{NH_4^+}$	3.67	0.36
C_{M^+}	0.00	4.00
$2C_{SO_4^{2-}}$	4.00	0.30
$C_{NO_3^-}$	0.27	2.74
C_{Cl^-}	0.14	1.37

This result gives the approximate relative concentrations that are actually observed in the respective particle types. For example, they are consistent with the observations made in New York City by Patterson and Wagman (1977) (see Figure 11).

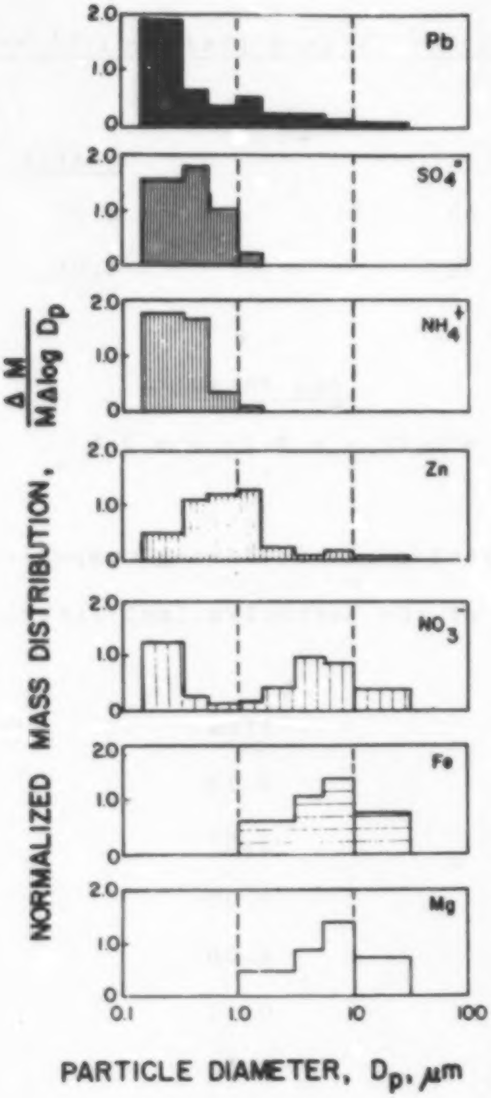


FIGURE 11. Normalized mass distribution functions of some species found in New York City aerosol. (From Sulfur Oxides, National Academy of Sciences, 1978)

Coarse, alkaline particles acquire nitrite from a reaction with nitric acid in the air. In addition, sulfite is formed in reaction with sulfur dioxide and is later oxidized to $SO_4^=$. If, in these reactions, an excess of acid is produced, it will react with ammonia forming ammonium ion. Fine sulfuric acid droplets formed intitially through photochemical oxidation of sulfur dioxide will grow and, through turbulent diffusion, reach ground-level air layers meeting a flow of ammonia. The degree of neutralization will consequently be dependent on available ammonia.

SUMMARY AND CONCLUSIONS

A variety of chemicals released into the atmosphere in the utilization of fossil fuels in mobile and stationary sources undergo complex series of chemical transformations. One reaction sequence leads to photochemical smog, which contains a number of potentially irritating and toxic products. Aliphatic hydrocarbons from mobile sources react with nitrogen dioxide in the presence of sunlight, and the various reaction sequences lead to the formation of aldehydes, ketones, ethers, olefins, and a series of nitrated organics. These reactions also lead to the formation of ozone, sulfuric acid, nitric acid, and highly reactive free radicals. Some of these products are formed as submicrometer-sized light-scattering aerosols.

As long as there are sufficient hydroxyl, hydroperoxy, and other radicals present to continue the production of nitrogen dioxide, quasi-stable materials including the above will form.

Most of these reactions occur during the daylight hours. However, some portion of the reaction and accumulation process will continue overnight if isolated from surface scavenging reactions. The photochemical smog process is an example of a synergistic physical-chemical process, which can form toxicants that expose and affect populations at distances far downwind.

The photochemical reaction products arising from mobile source precursors oxidize the sulfur dioxide released from both mobile and stationary sources, leading to the formation of submicrometer-sized sulfuric acid droplets via gas-phase reactions. The sulfur dioxide oxidation can also take place by catalytic reactions within droplets or at adsorption sites on carbon particles.

These chemical transformations are of great importance in the consideration of the health and environmental effects of air pollution. Secondary pollutants such as ozone and sulfuric acid are much more likely to produce physiological effects in exposed populations than any of the primary pollutants. The conversion of the pollutants to submicrometer aerosols increases their atmospheric residence times and downwind spread and is responsible for visibility degradation and acidic deposition in distant downwind regions. Since photochemical smog occurs on an episodic basis, the observed concentrations of the individual compounds will increase simultaneously. Ozone has been observed during episodes at concentrations that range to more than 300-1000 $\mu g/m^3$ (1-hour average), whereas "sulfate" has been observed at 25-60 $\mu g/m^3$ (24-hour average).

The primary and secondary pollutants can react with other chemicals within the source streams and within the general atmosphere. For example, trace metals and carbon within the primary ash particles can contribute to the formation of stable sulfur (IV) aerosols, which may have toxicological significance. Acidic droplets react with the ammonia in the atmosphere, which originates from biological decay processes at the earth's surface. Neutralization with ammonia reduces the toxicity of the aerosol and its impacts on forest and freshwater ecosystems. At present, it is not clear what ratios of hydrogen ion to ammonium ion can be expected in a community atmosphere, because the source terms for ammonia are not well-understood. However, sulfuric acid persists longer in rural-forested areas than in cities or agricultural locations.

A very large research effort over the past 30 years has led to a fairly comprehensive, but still incomplete, understanding of the processes that lead to photochemical oxidant formation. Significant effort to understand the pathways and kinetics of sulfur dioxide oxidation to sulfur trioxide and the neutralization of sulfuric acid by ammonia began much later, and our current knowledge remains incomplete. Reactions within droplets of H^+, NH_4^+, $SO_4^=$, NO_3^-, and Cl^- and exchange of ammonia, nitric acid, and hydrochloric acid between droplets and the surrounding gas phase have helped to explain the differing compositions of fine and coarse mode aerosols. Although significant information gaps remain in our understanding of oxides of nitrogen and sulfur within aerosols, we know considerably more than we did only a few years ago. We are on our way to developing a much clearer perspective of this chemical-physical complex.

This discussion has been limited to chemical-chemical interactions of fossil-fuel-derived air pollutants and chemicals in the ambient atmosphere. It illustrates that our knowledge of these interactions remains incomplete, despite the importance of these reactions to human health and welfare and despite a great deal of intensive research efforts over the last few decades. In intermediate pollutant situations, the complexity of the system frequently necessitates controls at the commercial, industrial, and utility sources rather than at locations within and surrounding the impact area. In this approach, a careful analysis of the potentially toxic pollutant exposures and a consideration of the available control technology lead to a proper judgment as to which precursors can be most effectively reduced by available technology at an acceptable cost.

The long atmospheric lifetimes of some aerosols and gaseous species make the problem of reducing all potentially hazardous exposures quite difficult. Each investigation has to identify all the widely dispersed sources and then determine their fractional contribution to the atmosphere. This approach would also be appropriate for chemical interactions in other media, especially those associated with groundwater and chemical dumping problems. The regional extent of an individual problem may not be as ubiquitous as it is for smog. However, it will usually be very difficult and time consuming to define accurately the precursor sources of potentially harmful products. Furthermore, once this is accomplished, chemical modeling of the affected systems will be even more difficult since the kinetics of most of the possible reactions are usually unknown.

In the case of the atmosphere, the role of free radicals in sulfur oxide chemistry has been identified only in recent years. The chemical interactions in water and waste problems are of great interest and important, but it will be much more difficult to describe these in general terms. This is because (a) the trace chemical composition in the waters, in the suspended solids, and in the soil vary greatly from location to location, and (b) the chemicals discharged into surface waters and/or placed in landfills are as varied in composition and physical form. Such interactions need to be considered on a case-specific basis.

ACKNOWLEDGMENTS

This review of some of the more important chemical interactions within the atmosphere draws heavily on the work of many others. In particular, the review of photochemical smog formation was based in large part on the 1977 National Academy of Sciences report *Ozone and Other Photochemical Oxidants* and on the 1978 U.S. Environmental Protection Agency publication *Air Quality Criteria for Ozone and Other Photochemical Oxidants*. The review of atmospheric transformation of the sulfur oxides is based primarily on (a) the January 1981 EPA external review draft of the *Air Quality Criteria for Particulate Matter and Sulfur Oxides*; (b) the paper by Charlson *et al*. (1978) entitled "Chemical Properties of Tropospheric Sulfur Aerosols"; and (c) the paper by Brosset (1980) entitled "Types of Transport Episodes in Northern Europe."

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Fate of Lipids and Other Organic Compounds in Aquatic Environments

S.C. Brassell and G. Eglinton[1]

This manuscript should serve as a framework for discussion, not as a detailed review of the subject, for which there exist adequate treatments (see Albaiges, 1980; Bolin *et al.*, 1979; Daumas, 1980; Duursma and Dawson, 1981; Eglinton, 1975; Faust and Hunter, 1971; Holdgate and White, 1977; Hutzinger, 1980; Hutzinger *et al.*, 1977; Morales, 1979; National Academy of Sciences, 1971; National Science Foundation, 1972; Raiswell *et al.*, 1980). The main theme draws on our own experience in exploring the natural lipid content of aquatic bottom sediments. Our concern here is with the likely long-term impact of multichemical exposures in the environment. In particular, when compounds are released into the environment, how do they affect the site or ecosystem into which they are introduced, how do they interact with it? What physical and chemical pathways do they follow, and what is their ultimate fate?

Figure 1 depicts the complexity of aquatic systems in terms of the typical sources of organic compounds in the environment. In particular, there are autochthonous contributions from sources within the environment under consideration and allochthonous inputs of compounds introduced by one of a number of transportation mechanisms. Both may be natural or anthropogenic in origin. Present knowledge of the natural and pollutant inputs and the

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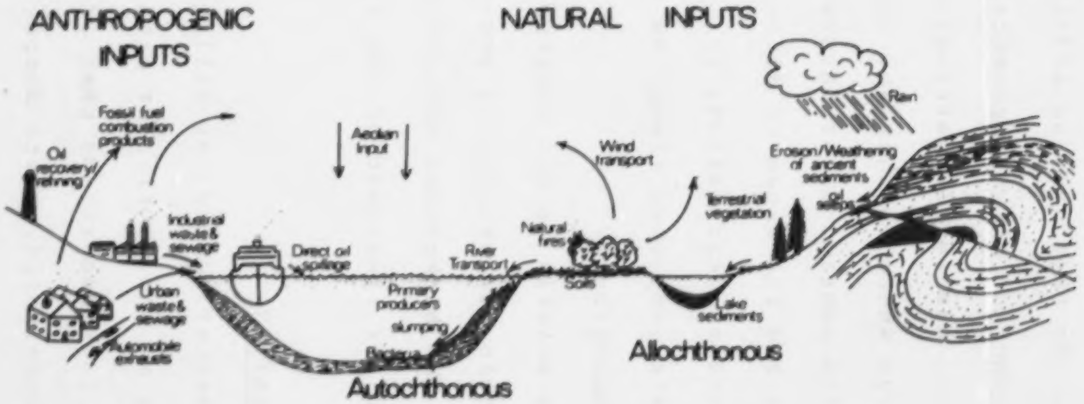
ANTHROPOGENIC INPUTS
NATURAL INPUTS
Fossil fuel combustion products
Oil recovery/ refining
Aeolian Input
Industrial waste & sewage
Direct oil spillage
Urban waste & sewage
Automobile exhausts
Primary producers
slumping
Bacteria
River Transport
Natural fires
Soils
Wind transport
Terrestrial vegetation
Lake sediments
Erosion/Weathering of ancient sediments
oil seeps
Rain
Autochthonous
Allochthonous

FIGURE 1. Pollutant and biogenic and other natural inputs of organic compounds to aquatic sediments. This sketch illustrates sources of organic compounds and their routes of transport to aquatic sediments. The major anthropogenic inputs are at the left of the scheme. These pollutants include the products from combustion of fossil fuels, inputs of sewage, industrial waste, oil spillage, etc. The autochthonous and allochthonous natural inputs are in the middle and to the right of the diagram, respectively. The autochthonous contributions to sediments include inputs from primary producers, especially phytoplankton, zooplankton, and the bacteria inhabiting the water column and sediment. Allochthonous contributors of organic matter include both thermally mature (e.g., oil seeps) and immature (e.g., shales, brown coals) sources of indirect input. In addition, contributions from terrestrial vegetation and soils, which act as a reservoir of organic matter, comprise an integral part of the allochthonous component. Combustion products of natural origin, such as those generated by forest fires or spontaneous burning of oil shales, oil seeps, etc., are further contributors of allochthonous organic compounds. In the environment, allochthonous and pollutant organic matter is transported by a variety of mechanisms, especially potamic and aeolian means. Slumping and, in some instances, ice rafting, can also be important agents in carrying organic matter. (From Brassell and Eglinton, 1980)

pathways of compounds within such systems is advanced to varying degrees; some processes are better understood than others. We aim herein to discuss in detail specific concepts within environmental science that bear on multichemical contamination, with particular reference to the natural system. To this end, we address some major questions: Concerning the introduction of multichemical contamination into an environment, how far do particular types of compound transfer and migrate, and where do they and their derived products reside? Is the migration continuous or intermittent, and does it finally terminate? What are the fates of different compound types in terms of their preservation, degradation, or mineralization? Can anthropogenic compounds always be separated and distinguished from the natural background?

In this paper, we refer only briefly to the original literature for illustrative purposes, as we do not propose to provide an extensive review of the topic. The background references cited in the first paragraph contain a fuller treatment of the broader aspects of the subject.

DISTRIBUTION, TRANSFER, AND MIGRATION

The fate of any compound, whether natural or pollutant, in the environment depends considerably on the particular ecosystem to which it is introduced. The various ecosystems that are environmentally significant include soils, waste dumps, lakes, rivers, estuaries, coastal regions, continental shelves, and oceans (deep sea). All are nonuniform and can, therefore, be further subdivided; for example, the water column and the bottom sediments of aquatic ecosystems are separate entities, possessing their own distinct flora and fauna. Similarly, there is a definite hierarchy of organisms in soils, each group colonizing a discrete horizon of the soil profile.

The ultimate distribution of chemical contaminants can be worldwide but, for convenience, we may consider their dispersal as local, regional, semiglobal, or global. Indeed, many studies focus on these geographical ranges; for example, one might investigate a specific oil spill as a local event or examine the problems of oil pollutants within the Mediterranean Sea on a regional basis. The semiglobal category is of particular importance with respect to the atmospheric and oceanic dispersal of chemicals within the southern and northern hemispheres because they act as virtually independent systems, having only a small flux of air and water masses between them. The latitudinal distribution of aerosol halocarbons, which originate principally in the northern hemisphere, well illustrates such differences between the two hemispheres (Figure 2).

Following its introduction into the environment, a chemical can transfer between media or migrate within an individual medium. Many agencies may be involved in such transfer or migration, but it is useful to distinguish the action of biota from physicochemical processes. Biological mechanisms of transfer involve organisms and food webs (notably their hierarchy of predation), whereas physicochemical processes involve winds (aeolian transport), water (groundwaters, rivers, ocean currents, rain, etc.), or sediment movement (slumping, turbidities, etc.). At a more detailed level, clay particles and other particulate materials play an important role in the transfer and migration of compounds in the aquatic

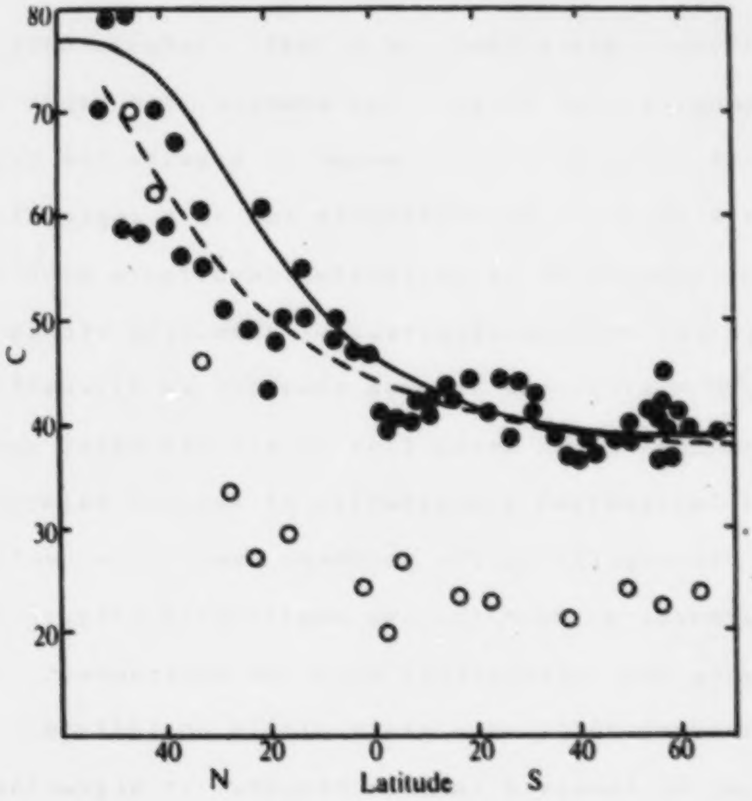

FIGURE 2. Distribution of trichlorofluoromethane in and over the North and South Atlantic Ocean. Key: ● = aerial concentrations (x 10^{-12}) by volume, o = seawater concentrations (x 10^{-12}), as aerial concentrations in equilibrium with water. Solid line = theoretical prediction; broken line = best fit third degree polynomial. (From Lovelock _et al_., 1973.)

environment, particularly where suspended sediment concentrations are high, as in rivers (Table 1). Similarly, zooplankton fecal pellets are an example of biological transfer mechanisms, whereby a capsule of phytoplankton debris is rapidly transported to the bottom sediments. In many instances, the overall processes of transfer and migration involve the constant interplay of biological and physical transport.

CONCEPTS OF SOURCES AND SINKS

Ecosystems are dynamic; they receive chemical inputs and release them to other dynamic ecosystems or to sinks. The interaction of a sink with the environment is usually controlled by kinetic factors (Figure 3); indeed, one can consider the presence of a chemical contaminant in the environment in terms of its residence time. The sink itself is normally either (a) temporary and subject to remobilization; (b) semipermanent and subject to remobilization after a reasonable length of time (e.g., polar ice caps); or (c) permanent, from which compounds are not released (but see below).

Temporary sinks are effectively compound reservoirs that vary in size, type, and duration. As such, they are inevitably a future source for the compounds, although perhaps in an altered form (see below). Two important varieties of temporary sinks that often interact are living organisms and sediments. For example, in aquatic systems the benthos can often rapidly remobilize sedimentary materials when the bottom waters become oxygenated.

TABLE 1

Ranges of Suspended Sediment Concentrations Common in Different Aquatic Environments[a]

Environment	Concentration (mg/liter)
Rivers	$1 - 10^5$
Estuaries	
Surface	$10^{-1} - 10$
Bottom	$1 - 10^3$
Shelves	
Surface	$10^{-1} - 10$
Middle	$10^{-2} - 1$
Bottom	$10^{-1} - 10$
Oceans	
Surface	$10^{-3} - 1$
Middle	$10^{-4} - 10^{-2}$
Bottom	$10^{-3} - 10^{-1}$

[a] Compiled from various sources by Kranck, 1980.

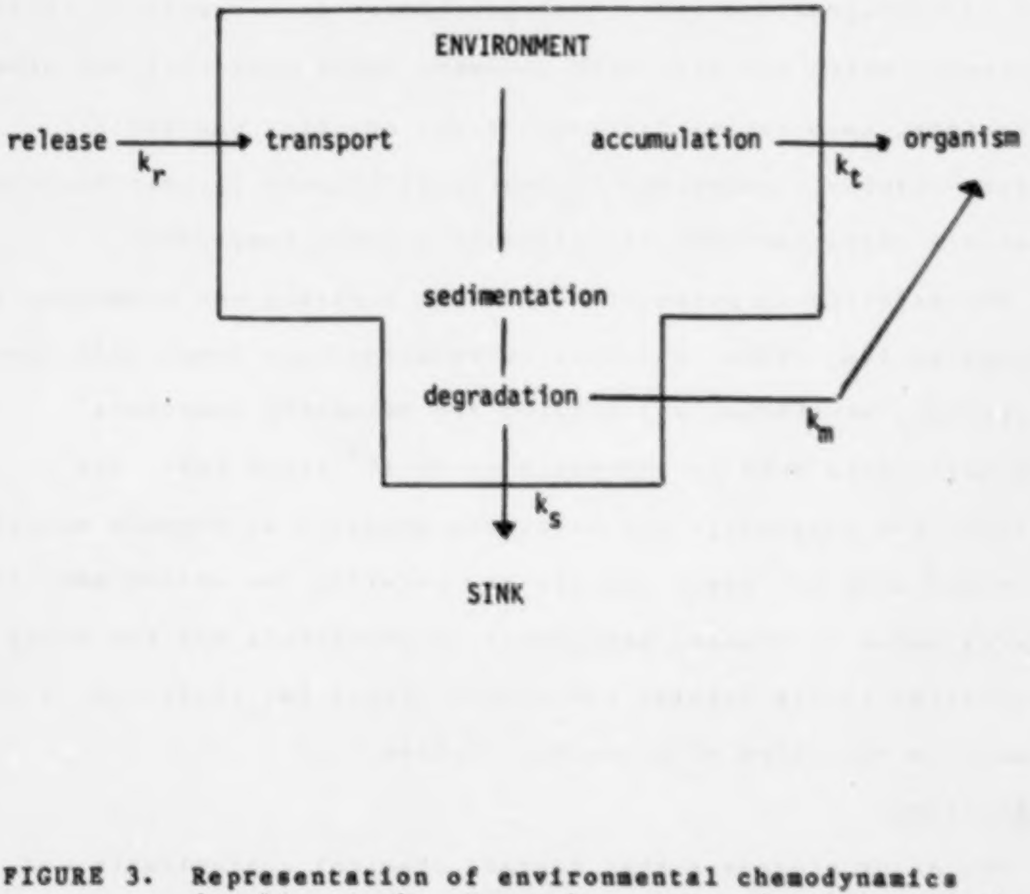
ENVIRONMENT
release
k_r
transport
accumulation
k_t
organism
sedimentation
degradation
k_m
k_s
SINK

FIGURE 3. Representation of environmental chemodynamics (ecokinetics). Key: k_r = rate of release of chemical; k_t = rate at which chemical (toxicant) becomes available to organism; k_m = same for metabolite; k_s = rate of disappearance of chemical (sink). (From Hutzinger et al., 1978)

Whether a compound is removed from the environment by sedimentation or recycled within the system may depend greatly on microbial populations. In particular, whether microbial systems are aerobic or anaerobic will influence the fate of the organic matter, especially its degradation pathways (Figure 4). In Figure 4, organic molecules, both natural and pollutant, may represent the electron acceptor (X) and therefore undergo microbial metabolism, such as hydrogenation and dehydrogenation. As a result of these processes, which may vary with sediment redox potential and other parameters, each successive zone, e.g., aerobic, anaerobic sulfate-reducing, anaerobic fermentation (Figure 5), may therefore possess a characteristic signature of organic compounds.

The majority of permanent sinks are probably not permanent in a geological time frame, but only in relation to a human life-span. At present, weathering and erosion are releasing compounds originally laid down in sediments up to 10^9 years ago. For example, the diagenetic and catagenic products of organic matter deposited some 10^7 years ago are now entering the environment in natural seeps of Miocene petroleums in California and are being redeposited in the coastal sediments. There is, therefore, a cyclic element in the roles of sinks and sources.

DEGRADATION

The major processes that degrade chemical contaminants and natural compounds in the environment include (a) physicochemical processes, where changes occur in response to different environmental conditions, such as temperature and pressure; (b) photochemical processes, which effect changes by the action of

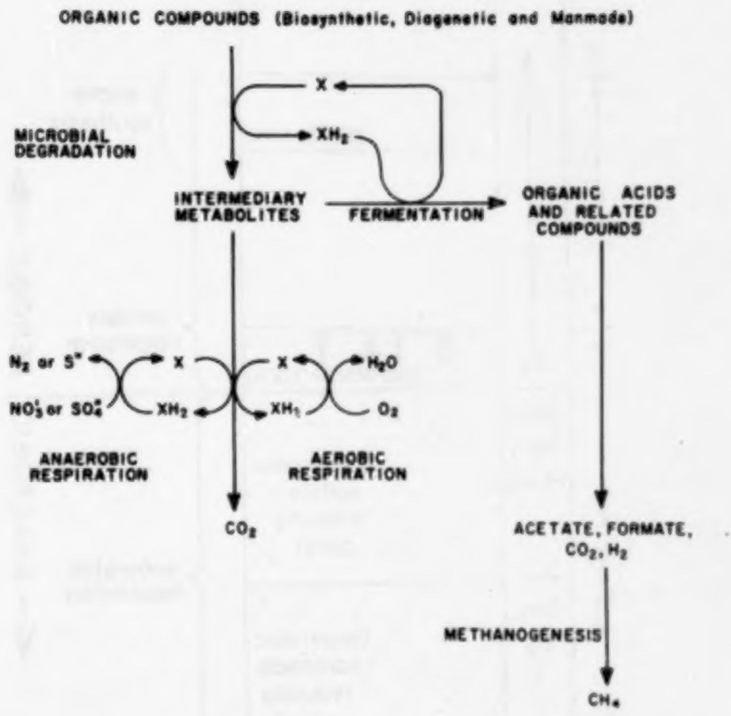
ORGANIC COMPOUNDS (Biosynthetic, Diagenetic and Manmade)
X
XH_2
MICROBIAL DEGRADATION
INTERMEDIARY METABOLITES
FERMENTATION
ORGANIC ACIDS AND RELATED COMPOUNDS
N_2 or $S^=$
X
X
H_2O
NO_3^- or $SO_4^=$
XH_2
XH_2
O_2
ANAEROBIC RESPIRATION
AEROBIC RESPIRATION
CO_2
ACETATE, FORMATE, CO_2, H_2
METHANOGENESIS
CH_4

FIGURE 4. Central reactions in microbial metabolism; X = electron acceptor. (From Gibson, 1980)

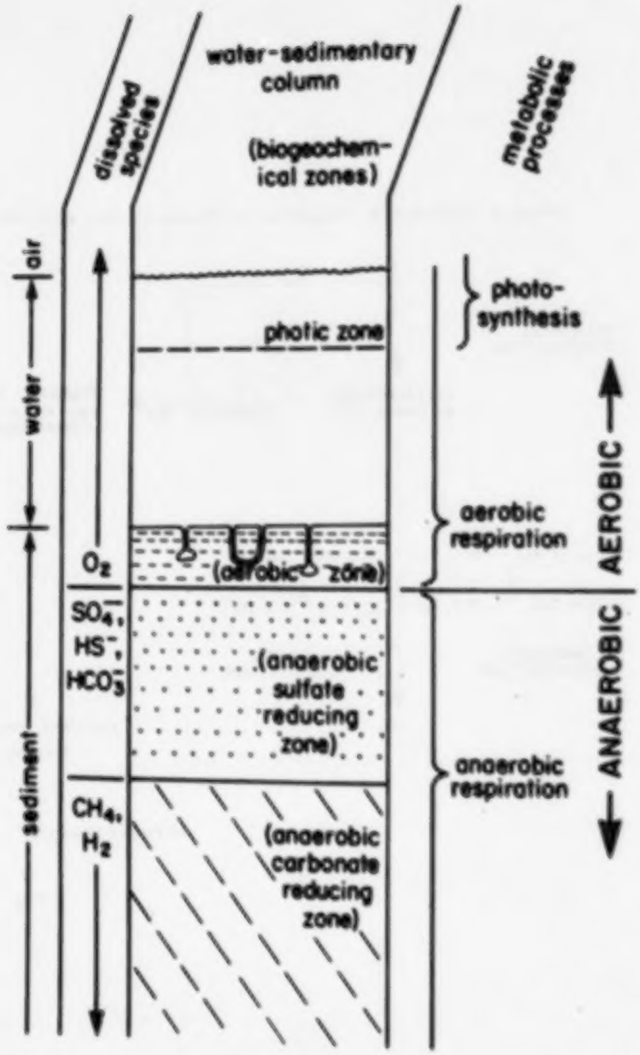
water-sedimentary column
(biogeochemical zones)
dissolved species
metabolic processes
air
water
sediment
photic zone
photo-synthesis
aerobic respiration
AEROBIC
ANAEROBIC
O_2
(aerobic zone)
$SO_4^{=}$, HS^-, HCO_3^-
(anaerobic sulfate reducing zone)
CH_4, H_2
(anaerobic carbonate reducing zone)
anaerobic respiration

FIGURE 5. An idealized vertical profile through a marine organic-rich sediment showing different reducing zones that develop as a consequence of microbiological succession. (From Claypool and Kaplan, 1974)

light; and (c) biological processes, wherein microbes cause the changes. These processes are accompanied by marked changes in the relative abundance of compounds, resulting from differences in the rates of their degradation and from the preferential degradation of specific components.

As mentioned above, the degradative pathways are greatly influenced by the oxygen content of the environment (Figure 4). In general, the anaerobic organisms that live in anoxic environments are less efficient in degrading organic matter than their aerobic counterparts; thus, even labile compounds may survive under such conditions. In oxic environments, biological reworking is enhanced (a) by the presence of animal scavengers at the sediment-water interface; (b) by worms, whose perturbation of the soil facilitates the diffusion of oxidants (e.g., oxygen, sulfate) within the sediments; and (c) by the smaller degree of organic complexation with toxic metals. In contrast, biological reworking is slowed in an anoxic environment (a) by the absence of animal scavengers; (b) by the restricted diffusion of oxidants into the undisturbed sediments; and (c) by the lesser utilization of lipids by anaerobic bacteria. One result of these differences is the markedly better preservation of organic matter in anoxic environments.

SEDIMENTARY PROCESSES

The process of sedimentation involves a complex interplay of many factors, all functions of the environment. The materials sedimented usually include a variety of autochthonous and allochthonous inputs of organic and inorganic species brought together by various means of transport, such as particulate sinking and turbidity currents. At the sediment-water interface, microbial populations are often high, recycling certain compounds within the biomass (see below) and aiding the incorporation of others into the sediment. In addition, changes in oxygen levels in the sediment can lead to a remobilization of its organic matter, as microbes capable of degrading organic compounds colonize the new environment. Clay particles bring adsorbed species to the site of deposition and also may protect them from microbial attack. The rate of sediment accumulation helps to determine the time that deposited species are exposed to the microbially active sediment-water interface and therefore affects the degree of preservation of the organic matter.

The settling rates of particles within water columns are an important facet of sedimentation processes. In essence, the larger, heavy particles, such as fecal pellets, sink faster and therefore are subjected to less degradation by external forces within the water column, although they often are an attractive body for microbial populations. Overall, the nature of a sediment (e.g., its oxygen content) and its overlying water column plays a major part in regulating the potential of sediments as sinks for chemical contaminants.

THE FATE OF COMPOUNDS

Compounds may survive in the environment unaltered, partly altered or degraded, or completely remineralized (Brassell and Eglinton, 1980). Compounds that can pass unchanged through the food web and occur intact in sediments include *n*-alkanes and sterols. The *n*-alkanes characteristic of pasture grasses survive in cow dung, as do those of insects in bat dung (Des Marais _et_ _al_., 1980). In the marine system, phytoplankton sterols can pass though zooplankton guts unaltered, as they occur in fecal pellets (Volkman _et_ _al_., 1980). In addition, halocarbons are chemical contaminants that can escape degradation in the environment (Figure 2).

Compound functionalities (double bonds, carboxylic acids, and hydroxy groups, etc.) are often the first part of a molecule to be degraded in the environment. The processes are varied, but they do not affect, initially at least, the basic molecular skeleton. For example, the products of sterol degradation include steroidal ketones and alkenes, which are only modified in their A and B rings; their side chains are generally unaffected, although several classes of organisms (e.g., sponges and coelenterates) do degrade them. A second example is the preservation of the phytyl side chain of chlorophyll produced by photoplankton as the pristane stored by the predator copepods (Blumer _et_ _al_., 1963). Compounds can also be degraded by anthropogenic processes. For example, the use of chlorine and ozone in water treatment generates anthropogenic components from the degradation of humic materials in natural waters.

Complete remineralization of compounds in the environment is best seen from carbon-14 (^{14}C) radiolabeling and carbon-13 isotopic studies ($\delta^{13}C$). For example, the liberation of $^{14}CO_2$ from the incubation of a ^{14}C-labeled lipid in a sediment provides direct evidence for such degradation, as do carbon-13 isotopic studies on the lipids of animals and insects feeding on C-3 and C-4 plants (Fry _et_ _al_., 1978; Jones _et_ _al_., 1979; Des Marais _et_ _al_., 1980). In many instances the breakdown of a compound leads to reincorporation of its carbon into the biomass. Such a process is clearly important in an oceanic water column, where the downward flux of organic matter represents perhaps only 5% of that in the euphotic zone.

EXAMPLES

Hydrocarbons are an important class of organic compounds that can originate from both natural and pollutant sources. Many processes influence their complex cycling and distribution within the environment, as depicted in Figure 6. Furthermore, hydrocarbon binding and clathration in the natural system are poorly understood processes that clearly influence the validity of analytical assessments of hydrocarbons in the environment. In general, the processes that act on the natural components will also affect pollutant compounds and may therefore lead to their concentration in particular environmental niches.

The oil seeps of the present and past provide one example of natural multichemical contamination. Hydrocarbons originating from such sources have been recognized in sediment trap particulates (Crisp _et al_., 1979) and bottom sediments (Simoneit and Kaplan, 1980) from the California coast and are therefore an important feature of that ecosystem. Such inputs have a parallel in anthropogenic oil spillage today, which can be recognized from characteristic fingerprint patterns of hydrocarbons in the environment (Brassell and Eglinton, 1980).

In many instances, the anthropogenic components may be superimposed on a natural signal, although their different chemical signatures make them readily distinguishable (Brassell _et al_.,

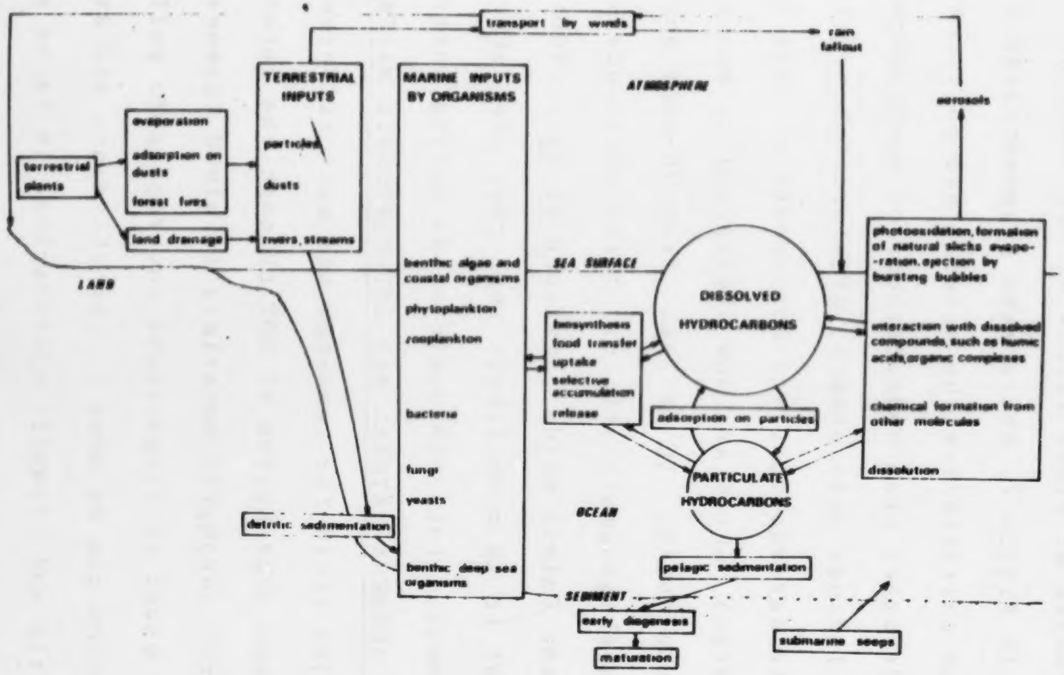
transport by winds
rain fallout
aerosols
ATMOSPHERE
terrestrial plants
evaporation
adsorption on dusts
forest fires
land drainage
TERRESTRIAL INPUTS
particles
dusts
rivers, streams
MARINE INPUTS BY ORGANISMS
benthic algae and coastal organisms
phytoplankton
zooplankton
bacteria
fungi
yeasts
benthic deep sea organisms
LAND
SEA SURFACE
DISSOLVED HYDROCARBONS
biosynthesis
food transfer
uptake
selective accumulation
release
adsorption on particles
PARTICULATE HYDROCARBONS
photooxidation, formation of natural slicks evapo-ration, ejection by bursting bubbles
interaction with dissolved compounds, such as humic acids, organic complexes
chemical formation from other molecules
dissolution
OCEAN
detritic sedimentation
pelagic sedimentation
SEDIMENT
early diagenesis
maturation
submarine seeps

FIGURE 6. Processes involved in the cycling of natural hydrocarbons in the marine environment. (From Saliot, 1981)

1978). For example, in the sediments of various lakes from Washington State, increasing amounts of oil-based pollutants gradually mask the natural hydrocarbon profiles of terrestrial higher plant waxes (Wakeham, 1976), but never completely obscure them. An example of mixed inputs of hydrocarbons to a sediment is illustrated in Figure 7, where mass fragmentography is used to recognize and characterize the natural and pollutant inputs.

Compounds other than hydrocarbons of anthropogenic origin in the environment include halocarbons, dichlorodiphenyltrichloroethane (DDT) and its derivatives, and components of polychlorinated biphenyls (PCBs), which are now widespread in biota and sediments throughout the world. It is less clear whether phthalates are of natural or anthropogenic origin. These compounds have been isolated from humic and fulvic acids (Lawrence *et al*., 1980), and may be biogenic, but it is more likely that they are anthropogenic and have been incorporated into the humic acids during their formation.

INTERACTION BETWEEN NATURAL AND ANTHROPOGENIC MATERIALS

This major field for research has many different aspects. One example is the degradation of DDT through the interaction of hematin and other iron porphyrin materials in blood tissues (Marei *et al*., 1978). The onset of large-scale anthropogenic pollution from fossil fuel combustion can be seen in the abundance and distribution of both aliphatic and aromatic hydrocarbons in dated sediment cores (e.g., Farrington and Tripp, 1977; Hites *et al*., 1980). Similarly, the downhole profiles of PCBs and two degradation products of DDT -- dichlorodiphenyldichloroethane (DDD) and 1,1-dichloro-2,2-bis-

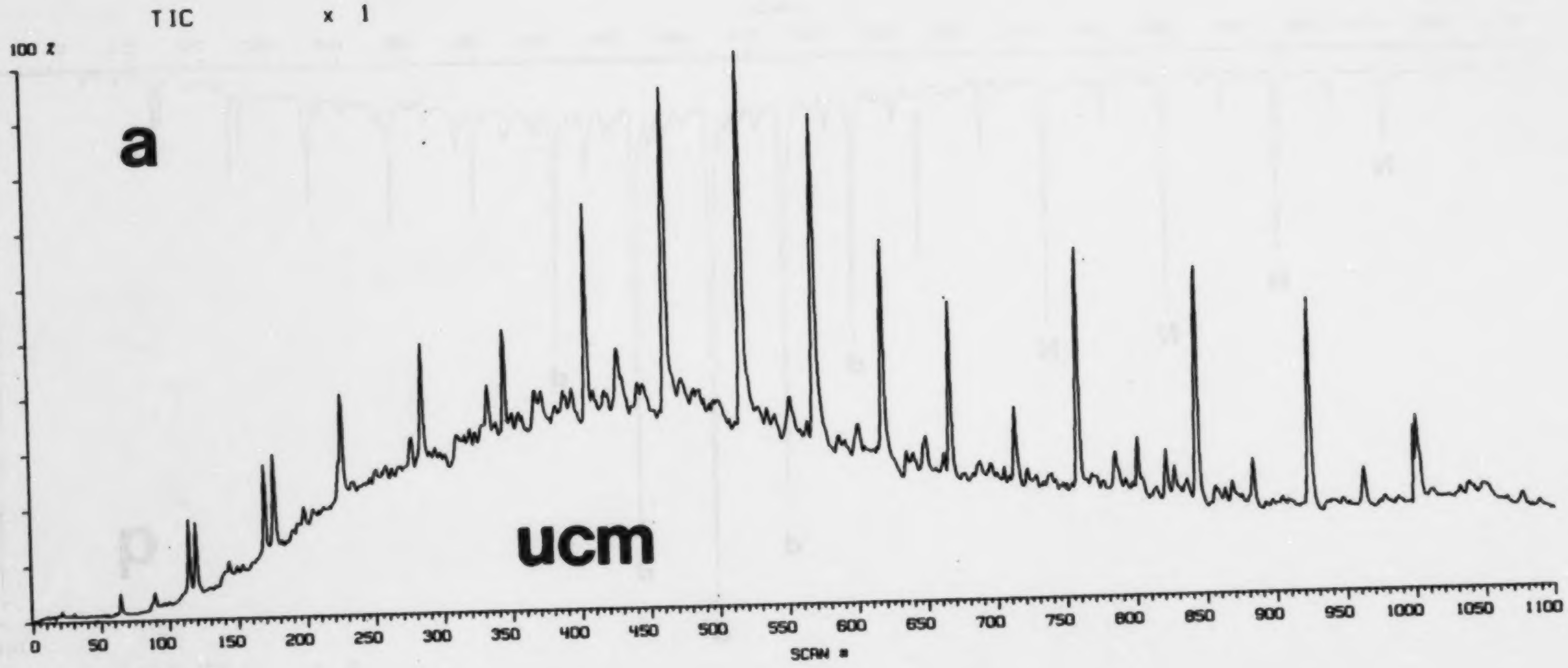

FIGURE 7. Selected gas chromatography/mass spectrometry data for an aliphatic hydrocarbon fraction from a surface sediment from ca. 4,000 m depth in the Mid-America Trench off Guatemala between Deep Sea Drilling Project Sites (DSDP) sites 494 and 497 (13°N, 91 W), illustrating components of natural (N) and pollutant (P) origin. Key: (a) TIC = total ion current. The unresolved complex mixture (UCM) underlying the major peaks is characteristic of biodegraded oils and therefore of pollutant origin. See (b) and (c) on next two pages.

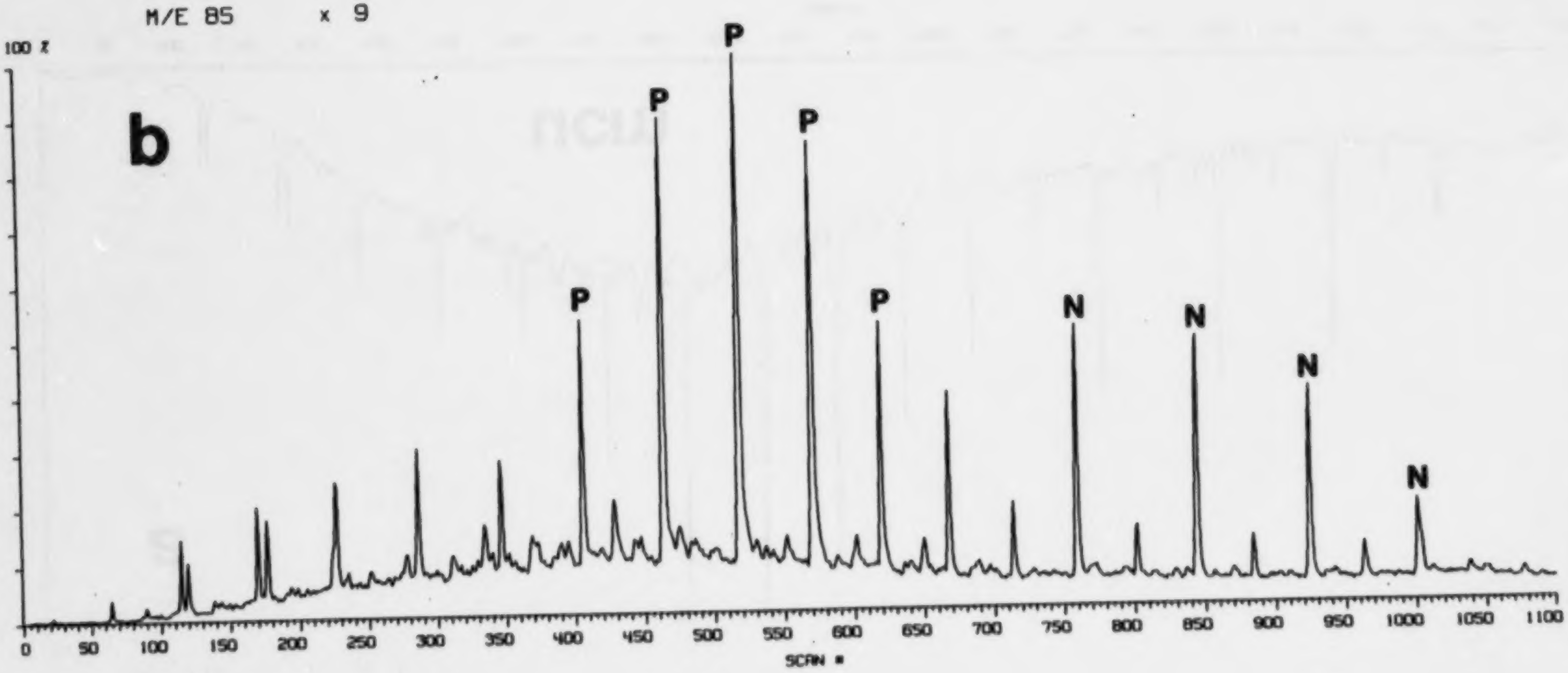

FIGURE 7 (Continued). (b) m/e 85 Mass fragmentogram. Restricted series of _n_-alkanes of pollutant and natural origin are evident, deriving from a refined or partly biodegraded oil and higher plants, respectively (Brassell _et al._, 1978).

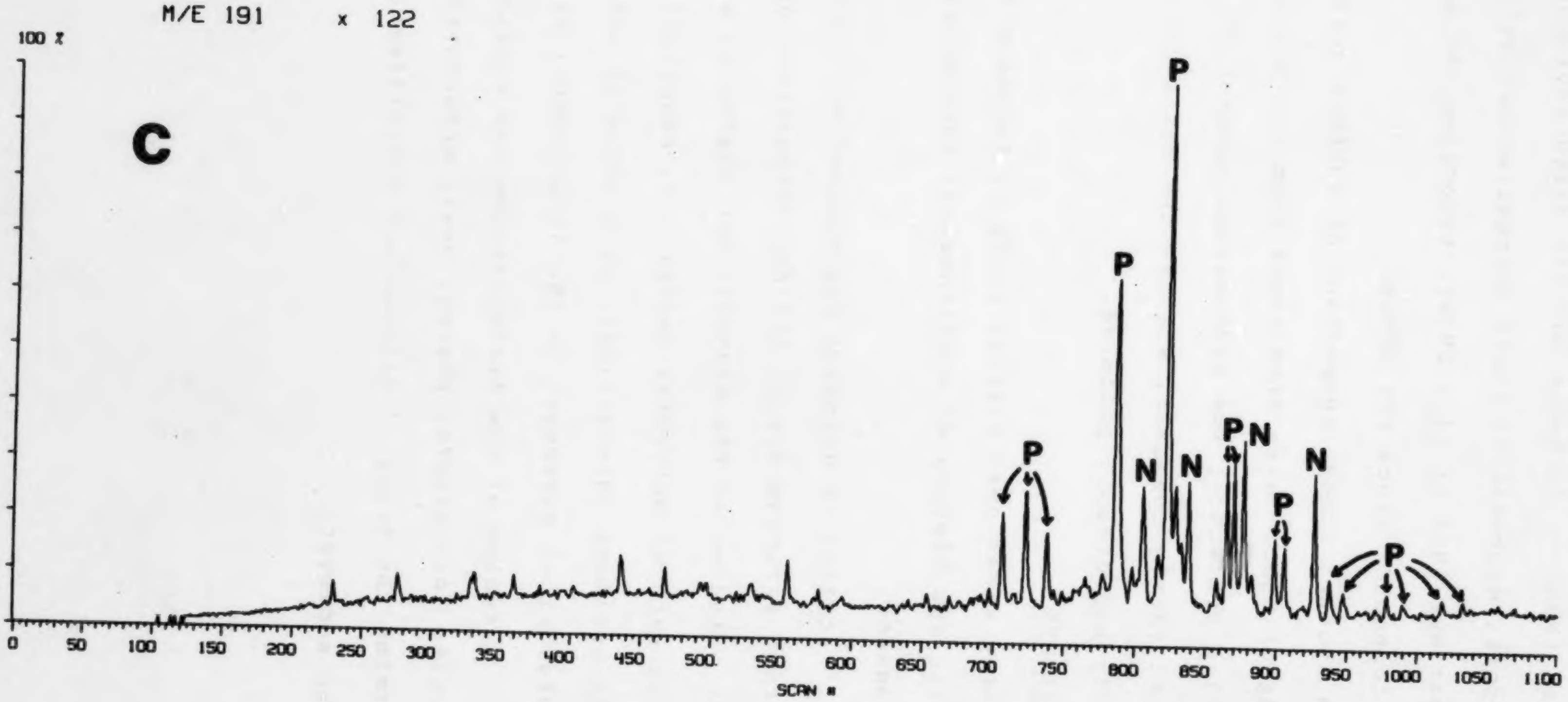

FIGURE 7 (Continued). (c) m/e 191 Mass fragmentogram. The thermally mature $\alpha\beta$-hopanes (C_{27} to C_{35}) are of pollutant origin, whereas the $\beta\beta$-hopanes and hopenes are derived from natural sources. (Brassell et al., 1978; Dastillung and Albrecht, 1976).

ethylene (DDE) -- in Santa Barbara Basin sediments show an exponential decrease in their concentrations with increasing sediment age (Hom *et al*., 1974), recording the growth in the use of these compounds since the 1940s.

In another study, comparison of surface sediments of three Washington State lakes with those from 0.5 m depth revealed selective pollution; the hydrocarbon content of one lake has remained fairly constant, whereas the other two environments are slightly and grossly polluted.

CONCLUSIONS

Three areas need further study to increase our understanding of the fate and history of multichemical contamination in the environment:

1. studies to document the natural molecular background in different environments to aid the recognition of pollutant materials;
2. studies of the transfer and migration and of the alteration of this natural molecular background, especially within the food web and in sediment interactions, as a means of understanding chemical and biological processes in the environment; and
3. studies of the incorporation and binding of organic molecules into mineral phases, humic structures, and kerogen to determine the rates of release and remobilization of such compounds in the sediment.

ACKNOWLEDGMENTS

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Multichemical Contamination: Assessment of Human Exposure

I.C.T. Nisbet[1]

The assessment of human exposure to chemical contaminants is an essential part of any risk assessment. This paper discusses factors controlling human exposure to chemical contaminants and points out difficulties in assessing the magnitude of such exposure. In keeping with the theme of this symposium, this paper is particularly concerned with problems that arise when general or residential environments are contaminated with poorly characterized mixtures of chemicals.

ROUTES OF HUMAN EXPOSURE

The three most important routes of human exposure to environmental contaminants are through inhalation, ingestion, or dermal absorption. Inhalation is frequently the most significant route. Volatile chemicals or aerosols may be released directly into the air from factories or during industrial accidents or fires, or they may infiltrate buildings from a landfill or from the subsoil and evaporate into the interior space (e.g., the incident at Love Canal). Sometimes building materials or consumer products are contaminated by materials (e.g., radon, asbestos or formaldehyde), which can be released into indoor air as vapors or dust. Volatile chemicals in domestic drinking water (e.g., chloroform) may evaporate when the water is boiled for cooking.

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[1] Clement Associates, Inc., Arlington, Virginia.

Ingestion can be an important route of exposure in certain circumstances. Drinking water can become contaminated through chlorination or the contamination of groundwater. Vegetables may be cultivated or domestic animals raised on land that has become contaminated through industrial accidents (such as that at Seveso) or through the use of contaminated sewage sludge. Food may be directly contaminated during processing or packaging (e.g., with polychlorinated or polybrominated biphenyls) or fish that are caught for food may be contaminated in surface waters containing persistent, bioaccumulated chemicals [e.g., methylmercury or 1,1-bis(p-chlorodiphenyl-2,2-dichloroethylene (DDE)]. Finally, ingestion of house dust may sometimes be a major route of exposure to babies (e.g., for lead or domestic pesticides).

Dermal absorption is usually not a significant route of human exposure to contaminants in the general environment. It may be important in cases where soil or water is heavily polluted with organic contaminants (e.g., polychlorinated biphenyls or tetrachlorodibenzo-p-dioxin) or where house dust or consumer products have high levels of chemicals such as pesticides or flame retardants.

FACTORS CONTROLLING HUMAN EXPOSURE

The factors influencing human exposure to chemical contaminants fall into two broad classes: those influencing the concentrations of the chemicals in various environmental media and those influencing the uptake of the chemicals by humans from these media. The first class includes factors characterizing the release of the chemical into the environment (quantities released, location, frequency, physical and chemical form, medium into which released, etc.) and those characterizing its transport and fate in the environment (persistence, reactivity, solubility, volatility, absorption to materials, uptake by plants and animals, etc.). The second class includes factors characterizing the amount of contact between the human population and contaminated substrates (number of people, age and sex distribution, activity patterns, breathing rates, consumption of food and water, etc.), those characterizing the absorption of the chemicals per unit of contact (absorption coefficients), and those relating the amount ingested to tissue doses (metabolism, tissue distribution, and excretion).

The potential for human exposure to chemical contaminants varies enormously according to the nature of the chemical, the circumstances in which it is released, and the characteristics of the exposed population. At one extreme, a chemical that is released directly into a drinking water supply will be transported efficiently to the human population. At the other extreme, a reactive chemical that is released into turbulent air in a sparsely populated area will have a very low potential for human exposure. Time-scales for human exposure also vary enormously, from a few minutes for reactive gases released accidentally into the atmosphere to decades or centuries for persistent chemicals released into groundwater.

ASSESSMENT OF HUMAN EXPOSURE

Assessment of the extent and magnitude of human exposure to multichemical contaminants may be conducted for several purposes:

1. to identify the number of people potentially exposed or the geographical extent of contamination;

2. to distinguish highly exposed groups, which would serve as the basis for epidemiological studies; or

3. to serve as the basis for risk assessments to determine whether or not the exposures pose health hazards.

For the first two purposes, semi-quantitative measures of exposure (merely distinguishing between highly exposed and less exposed persons) often suffice. However, most risk assessments require a comparison of estimated levels of exposure with levels of dosage known to pose hazards. Such cases necessitate quantitative estimates of exposure.

Although the usual purpose of conducting quantitative assessments of human exposure is for comparison with toxic dose levels, "exposure" is a more complex concept than "dose" and usually cannot be specified in a single measure. In the first place, humans are frequently exposed by more than one route; a proper specification of the magnitude of exposure should separate the contributions of each route. For inhalation and dermal absorption, the best measure of exposure is often the concentration of the substance in the ambient medium; the efficiency of aborption into the body may be poorly known or variable. In most real situations, exposure to chemical contaminants fluctuates markedly from day to day or even hour to hour. Although "average" levels of exposure may have some value for comparison with the constant dosages used in toxicological experments, in many cases transitory peak levels of exposure may be of much greater significance. Hence, a rigorous specification of exposure should include some statistical measures of temporal fluctuations. Finally, levels of exposure vary markedly from person to person, dependent on location, age, sex, occupation, habits, etc. A full specification of exposure should include measures of variability within the population, including estimates of exposure of sensitive subgroups.

A full description of population exposure to chemical contaminants may require a prohibitively large volume of data, especially if many chemicals are involved. Although techniques of sampling for chemical contaminants have often been discussed (see other papers in this workshop), it is unusual for a sampling program to be designed specifically to support a quantitative exposure assessment. For this reason, critical information on spatial and temporal fluctuations in exposure has rarely been obtained.

Most assessments of human exposure have been based primarily on ambient monitoring, which measures concentrations of the chemical in samples of air, water, soil, food, or other media. If the sampling is sufficiently extensive and systematic, these measurements can be used to calculate average concentrations and, in some cases, statistical measures of variability as well. Independent estimates are then made of the number of poeple in the exposed population, their demographic characteristics, the amount of time they spend in contact with the contaminated media (from surveys of human activity patterns), and their rates of intake (from measurements of breathing rates, water and food consumption, etc.).

To convert these data into measures of exposure, it is necessary to estimate the efficiency of absorption from each medium. This is often the most uncertain part of the assessment, and requires knowledge of the physiology of absorption, familiarity with the limited measurements on other representative chemicals, and (in some cases) informed guesswork. Depending on the extent of the data base, such an assessment may lead, at one extreme, to a detailed statistical description of exposures or, at the other extreme, to a single estimated number for "typical" or "maximum" levels of exposure.

EXPOSURE MODELS

In many, if not most, cases, some important items of information are missing, and it is necessary to fill the gaps by using exposure models. The models most commonly utilized in exposure assessment are those that are used to estimate ambient concentrations in the absence of sufficient measurements. These include atmospheric models of dispersion and fallout from point sources, hydrologic models of transport in groundwater and evaporation from surfaces, and ventilation models to predict concentrations of pollutants inside buildings or other enclosed spaces. Pharmacokinetic models are widely used, both to predict bioaccumulation of persistent chemicals in fish and to describe the distribution of toxic chemicals among target organs in the human body. Statistical models are often used to simplify the description of exposures; data on chemical contaminants often fit closely to log-normal distributions.

Although models are often very useful in characterizing and explaining patterns of exposure when extensive data already exist, they are of limited value as predictive tools in the absence of data. Exposure is controlled by many poorly understood factors, and critical elements (such as transfer coefficients) in the models are extremely difficult to predict.

When both monitoring data and a firm theoretical basis for calculating exposures are lacking, useful estimates can often be obtained by using analogies and surrogates. An analogy is helpful when the chemical of interest is physically and chemically similar to another whose exposure potential has already been studied. For example, knowledge of the environmental behavior of 1,1-bis(p-chlorophenyl)-2,2-dichloroethylene (DDE) was used to predict likely patterns of human exposure to polychlorinated biphenyls, and studies of occupational exposure to pesticides have been used to predict occupational exposure to other pesticides applied in a similar manner.

A surrogate is useful when two or more chemicals are released into the environment simultaneously; measurements of exposure to one can be used as the basis for estimating exposure to the others. For example, the concentration of benzo(a)pyrene has been used as a surrogate measure of the magnitude of exposure to air pollution, and exposure to nicotine has been used as a surrogate measure of the magnitude of exposure to cigarette smoke.

Assessment of human exposure by any of these methods is a lengthy process that requires large quantities of data to be done rigorously. Even if adequate data are available, there are often great uncertainties in the estimates of exposure, because of wide variability in ambient concentrations and lack of knowledge of absorption coefficients. In a limited number of cases, these uncertainties can be avoided by using the human population to monitor its own exposure. If a chemical is retained in human tissues for even a modest period (a few days or more), the concentration of the chemical in the tissues is a fairly direct measure of the individual's exposure. Tissue residue measurements have been used successfully to monitor human exposure to a number of pesticides (using residues in fat, blood, and urine), polychlorinated and polybrominated biphenyls (fat and blood), mercury and lead (blood), and arsenic (hair).

Where practicable, this technique has two major advantages: It provides a measure of total exposure via all routes, and it often provides a direct measure of concentrations in critical tissues, facilitating comparisons with toxic doses in experimental species. It has two offsetting disadvantages: First, it is usually impossible to identify which routes of exposure are more important. Second, although tissue residues provide an integrated measure of exposure over a period prior to sampling, the length of this period is usually unknown in the absence of specific pharmacokinetic studies. However, when it is practicable to obtain tissue monitoring data, this method usually provides by far the most reliable measures of human population exposure.

SPECIAL PROBLEMS RAISED BY MULTICHEMICAL CONTAMINATION

Cases in which the environment is contaminated by a poorly characterized mixture of chemicals (e.g., from industrial effluents or chemical landfills) raise several special problems in exposure assessment. Perhaps the most difficult ones are those of adequately sampling and characterizing the mixtures. Because these problems are discussed in other papers in this workshop, they will not be reviewed again here, except to point out the value of selecting appropriate surrogate chemicals and using them to characterize the distribution of contamination.

An important consideration in exposure assessment is that mixtures of chemicals may be rapidly differentiated as they are transported through the environment. For example, volatile components of mixtures are differentially transported across water-air or soil-air interfaces, and soluble components are differentially transported through soil-water matrices. Indeed, some parts of the natural environment act as giant chromatographs, and the principles of chromatography can be applied to estimate both the magnitude and the timing of exposures. The likelihood that different chemicals will display different spatial and temporal patterns of occurrence should be considered in designing sampling schemes.

In some cases, the environmental behavior of chemicals may be influenced significantly by the presence of other chemicals in complex mixtures. At Love Canal, for example, the presence of organic solvents probably made nonpolar contaminants such as lindane much more mobile in soil than they would have been in aqueous solvents. There is some evidence that rates of bioaccumulation and volatilization of polychlorinated biphenyls are strongly influenced by their constitution as mixtures. In a few cases, the presence of one chemical may strongly influence the biological uptake and retention of others; for example, exposure to endrin dramatically reduces the quantity of 1,1-bis(p-chlorophenyl)-2,2-dichloroethylene stored in the human body. Few such cases have been documented, but they are potentially significant in assessing human exposure to complex mixtures.

CONCLUSIONS AND RECOMMENDATIONS

1. Human exposure to chemical contaminants may take place by several routes and is controlled by many different factors.

2. Quantitative assessment of human exposure to chemical

contaminants requires large volumes of data. These requirements may be prohibitive if exposure to many chemicals must be assessed.

3. The use of analogies and surrogates is very desirable, and probably necessary, for adequate assessment of exposures to complex mixtures.

4. Sampling programs should be designed specifically to meet the data requirements for exposure assessment, including the need to assess spatial and temporal variability.

5. The use of human tissue-monitoring data offers important advantages for assessing exposure. Wherever practicable, human tissue monitoring for carefully selected surrogate chemicals should be the monitoring technique of choice.

PART III: TOXICOLOGICAL INTERACTIONS OF MIXTURES IN HUMANS AND LABORATORY MODELS

Toxicological Interactions of Mixtures in Humans and Laboratory Animals

Robert A. Neal[1]

The purpose of this paper is to suggest the toxicological procedures that should be performed to evaluate whether a significant health risk is posed by exposure of humans to a mixture of chemicals that may be present in a particular environment. There are four general types of chemicals to which humans may be exposed: organic chemicals, inorganic chemicals, suspended particulates, and radiochemicals. The most hazardous suspended particulate is asbestos; inhalation of this substance leads to adverse health effects in humans, including lung disease and the induction of pulmonary cancer. However, there is no current determination as to whether ingested asbestos poses a significant adverse health risk. The inorganic anions and cations of greatest health concern are nickel, arsenic, cadmium, chromium, lead, and mercury. Humans may occasionally be exposed to others, but, because of their biological properties, these elements are the most dangerous. The best method for controlling the potential adverse health risks posed by asbestos, inorganic chemicals, and radiochemicals involves periodic monitoring for the levels of these materials in the air, finished drinking water, and land surface areas.

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Evaluating the potential adverse health risks presented by organic chemicals is a much more complex task. The number and variety of compounds is enormous. For example, more than 1,000 organic chemicals have been detected in finished drinking water supplies in the United States.

The current interest, as well as the majority of the resources, of regulatory agencies within the United States has been devoted to searching for the presence of carcinogenic and mutagenic chemicals in the environment. However, chemicals may also produce teratogenic, acute, or adverse effects on various organ systems including the hematopoietic and nervous system, the immune system, or the reproductive system. Consequently, any protocol for evaluating the potential adverse health effects of a mixture of chemicals to which people may be exposed in a particular environment must account for all the possibilities posed by the organic and inorganic chemicals, suspended particulates, and radiochemicals present in that environment.

TOXICOLOGICAL EVALUATION OF MIXTURES

Since exposure to organic and inorganic chemicals, particulate matter, and radiochemicals may cause a variety of toxic effects including acute, subacute, and chronic diseases in humans, appropriate tests or monitoring systems must anticipate and search for these effects. The first step is to evaluate the chemical composition of the finished drinking water, air, and ground and building surfaces that may lead to dermal absorption in search of contaminants of toxicological concern. For compounds with an adequate toxicological data base, potential adverse health effects can be determined, and maximum exposure limits set. For those having an incomplete data base, we can use the suggested maximum exposure levels as an interim measure.

The second step is the oral or inhalation exposure of experimental animals to various doses of an artificial mixture of organic chemicals in concentrations proportional to those present in the environment. For one thing, the separation, identification, and toxicological evaluation of each of the organic compounds present may be an impossible task. Moreover, people in that environment will likely be exposed to the mixture of organic compounds and not to individual substances. Therefore, exposure of experimental animals to the mixture of organic compounds is a toxicologically sound approach, which will permit examination of their possible antagonistic, additive, and synergistic effects.

ACUTE TOXIC EFFECTS

The potential for acute toxicity in humans from exposure to inorganic chemicals in the environment can largely be determined by monitoring for inorganic ions known to produce such effects. The technology for monitoring for inorganic chemicals at levels that may pose a potential health risk is readily available (i.e., the atomic absorption spectrometry). Maximum concentration limits in water and threshold limit values in air have been set for most of the inorganic chemicals that produce toxic effects in humans by these routes. These limits, together with the appropriate monitoring, should provide the means of determining whether inorganic chemicals that may be present pose an unreasonable acute toxicity risk to people living in a contaminated area.

Similarly, the presence of an acute radiochemical hazard can be assessed by monitoring for radioactivity of various types (alpha, beta, and gamma) and comparing the results with the maximum radiological exposure limits for these kinds of radiation. To measure the potential acute toxic effects of the organic chemicals present, however, will require a combination of monitoring for known acutely toxic organic chemicals that may reasonably be present as contaminants plus exposing experimental animals to various doses of a representative mixture of organics. To the extent possible, the components of the artificial mixture should be in the same relative proportion present in the environment, as determined by analytical monitoring.

SUBCHRONIC HEALTH EFFECTS

As with acute toxic effects, the subchronic effects of organic, particulate, and radiochemicals are best controlled by monitoring and reference to maximum concentration limits and threshold limit values. For organic chemicals, subchronic testing of various doses of the artificial mixture of organics in experimental animals will provide the most useful information. Results of this test will indicate the ability of the mixture to produce adverse effects on various organ systems, the hematopoietic system, the nervous system, and the immune system.

CHRONIC HEALTH EFFECTS

Asbestos inhalation produces chronic disease in humans; the data on the incidence of tumors as a result of chronic ingestion of asbestos fibers contained in drinking water are conflicting and incomplete. However, because asbestos inhalation does produce cancer in humans, it is prudent to monitor for its contamination of drinking water.

The potential adverse health effects from chronic exposure to inorganic chemicals and radiochemicals present in a particular environment can best be estimated and controlled by monitoring for the levels of inorganic chemicals and radiochemicals known to have such effects in humans and enforcing the maximum concentration limits, threshold limit values, or radioemission limits. As to chronic effects of mixtures of organic chemicals in a particular environment, the major regulatory emphasis in the United States has been on their potential to produce an increased incidence of neoplasia in man. However, other chronic effects can occur, so the studies designed to search for potential health effects should consider all that may occur when humans are exposed to the mixtures. These include organ system effects, neurotoxicity, and effects on the hematopoietic and immune systems, as well as an increased incidence of neoplasia.

Since chronic studies in experimental animals with an artificial mixture of organic compounds are expensive, short-term tests should be conducted as a preliminary examination of the mixture for mutagenic and mammalian cell-transforming properties. However, in the final analysis, the results of the short-term tests must be verified in the standard rodent bioassay of the same mixture of organic compounds.

The artificial mixture of organic compounds from the particular environment should be examined for teratogenicity through the standard teratogenic tests in rodents. In addition, employment of the standard two-generation reproductive test in rodents will reveal the potential for adverse health effects on the rodent male and female reproductive system by administering the artificial mixture of compounds at the maximum tolerated dose and fractions thereof. Some tests should examine the effect of chronic administration of the mixture on the responsiveness of the immune system, and others will be epidemiological studies on the exposed population.

We must establish a monitoring program to gather data on the potential for the chemical contaminants to produce adverse health effects. This will include maintaining a registry of mortality data, cancer registry data, data on reproductive efficiency, congenital malformations, school absentee records, and hospital discharge records. Although data of this type will be difficult to interpret because of a number of confounding variables such as smoking, diet, and occupation, such information nevertheless should be gathered so that retrospective studies of the potential increased incidence of a disease might be correlated with exposure to the mixture of chemicals.

Although continuous monitoring for the occurrence of potential carcinogens in the particular environment may be possible using current short-term tests, similar relatively inexpensive tests for compounds producing reproductive toxicity, teratogenicity, and/or organ systems damage are not available. Therefore, analytical monitoring of the air, water supply, and surface areas is the best procedure for constantly seeking the appearance of new chemicals that may have adverse health effects.

LIMITATIONS IN ASSESSMENT BY THE PROPOSED METHODOLOGY

A major potential limitation in the proposed methodology for examining for adverse health effects of mixtures of chemicals in a particular environment is the ability of the chemists to provide the toxicologist a representative analysis of the organic, inorganic, and particulate material present. There are a number of technical problems to be overcome in deciding what artificial mixture of chemicals is representative of the exposures in that environment. Another problem is the unknown degree of interactions of the organic compounds in the mixture to be used in the animal studies, which may form products that were not originally in the particular environment because they were present in lower concentrations and less likely to react with each other.

Another limitation to the proposed tests, one that is inherent in toxicological testing for many purposes, involves extrapolation of the data from experimental animals to humans. This limitation will perhaps be most evident in the estimation of the potential carcinogenicity of the mixture of organic chemicals. Moreover, the regulatory agencies and the scientific community have not reached a consensus concerning the interpretation of the data from testing for mutagenic properties. Thus, although considerable progress has been made in recent years in developing the methodology for evaluating the potential mutagenicity of chemicals, the interpretation of the data relative to an adverse health effect in humans has lagged behind.

Toxicodynamics and Biotransformation

J.R. Withey[1]

The total additive toxicological effect following exposure to a number of chemicals in combination, either simultaneously or consecutively, is a problem that is now being addressed by governmental regulatory agencies and concerned lobby groups in various parts of the world. Two examples, from the North American continent will serve to illustrate the importance and timeliness of this workshop and perhaps allow some definition of the magnitude and nature of the concern.

A recent publication by the National Academy of Sciences (1980) addressed the hazards associated with maritime personnel exposed to multiple cargo vapors. The "special group at risk" would include not only the ship's crew but more especially, because of their wider responsibilities, the personnel of U.S. Coast Guard inspection teams.

Recently, the U.S. Environmental Protection Agency (J.F. Stara, personal communication, 1980) initiated a $1.7 billion program to study approaches to the problem of the cleanup of chemical dump sites (Project ATLAS). The first stage has been to evaluate the toxicology data base of selected compounds, known or believed to be present in seven or eight specific sites, and to document the present state of knowledge in the following 11 subjects areas: chemical and physical properties, environmental fate and transport, levels of exposure, disposition and pharmacokinetics, toxicity, teratogenicity, mutagenicity, carcinogenicity, synergism and antagonism, current regulations and standards, and effects of primary concern. Of particular, importance are the topics of synergism and antagonism, and of, disposition and pharmacokinetics. The following is a priority listing of those compounds considered so far.

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INORGANICS

Beryllium
Fluorides
Mercury
Arsenic
Barium
Nitrate/Nitrites
Chromium
Nickel
Silver

ORGANICS

Halomethanes
Pentachlorobenzene
Chlorofluorobenzenes(5)
Pentachloronitrobenzene
Xylenes
Chlorinated benzenes(4)
Chloroform
Phthalates
Chlorinated toluenes
Tetrachloroethylene
Toluene
Trichloroethylene
Trichlorobenzenes
Benzene
Chlorophenols
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin(TCDD)
Acrylonitrile
Ethylbenzene
Methyl chloroform
Methylene chloride
Vinylidene chloride
Carbon tetrachloride
Nitrophenols
Phenols

PESTICIDES

Arochlors
Chlordane
Hexachlorocyclohexane
Aldrin/Dieldrin
Endrin
Mirex
Dichlorodiphenyltrichloroethane (DDT)
Dichlorodiphenyldichloroethane (DDD)
1,1-Dichloro-2,2-bis-ethylene (DDE)
Heptachlor

There are very few examples in the literature that relate to the evaluation and interpretation of the mechanism for joint toxic action of more than two chemicals in combination. Of course, the logistics for such investigations have probably been one of the major impediments, since the number of different permutations and combinations increases rapidly with the number of individual species within the mixture. Moreover, the relative quantitative composition of the mixture is also a variable that will add to the complexity of a suitable experiemental design to evaluate the addtivity or non-additivity of the total induced effect.

If two substances, A and B, are administered as a mixture, where $\P_A$ is the fraction of the component A, and $1-\P_A$ is the fraction of component B, then the joint toxic action may be represented as

$$\frac{1}{L\ D\ \alpha(AB)} = \frac{\P_A}{L\ D\ \alpha(A)} + \frac{1-\P_A}{L\ D\ \alpha(B)}$$

where L D α(AB) is the dose of the mixture AB that is lethal to α% of the test population. For example, in the experimental design to evaluate the joint toxic action of a pair of miscible organic liquids (Withey and Hall, 1975), 6 different mixture compositions, tested at 5 different dose levels with 20 animals dosed at each level, required 600 animals.

In another and different kind of study (Gullino *et al*., 1956), which probably relates to the subject in question more closely, 10 essential amino acids required that 1,023 possible combinations be tested for only one set of composition ratios. The late Jerome Cornfield discussed this example at some length in his Presidential Address to the American Statistical Association, in a paper entitled "A Statisticians Apology" (Cornfield, 1975).

In the middle 1950s, scientists noted that adverse reactions frequently occurred when mixtures containing essential amino acids were administered to patients following surgery. Individual dose-response curves in rats had facilitated assessments of the individual toxicities, and it was considered that the response in humans was a consequence of a synergistic effect. Clearly, the testing of all possible combinations of the 10 essential amino acids represented a Herculean task, there being so many possible combinations. Gaddum (1953) had proposed an empirical approach to the testing of the additivity of joint toxic action, in terms of dose. Because the dose-response curves for each of the amino acids were so steep, an administered dose of any pair of amino acids, equal to one-half of the lethal dose to 99.9% of each, should have led to no more than a 2 to 6% mortality. In fact, 100% mortality occurred when one paired combination was tested in this manner. The next stage was to test a mixture of all 10 amino acids, each at a dose level of one-tenth of the respective lethal doses to 50%. No animals died, although a dose-wise response should have led to a 50% mortality. A 50%

kill occurred only when the amount of each ingredient was increased to 1.7 x 0.1 LD50.

When 10 separate mixtures were tested, each containing 9 amino acids at a dosage equivalent to one-ninth x 1.7 of their individual lethal dose to 50%, for all but 1 of the 10 mixtures the departure from additivity was the same magnitude as for the mixture containing all 100. The mixture lacking L-arginine was much more toxic than the rest, which immediately suggested an inhibitory or protective effect due to this specific amino acid. Further research revealed that the toxic effect responsible for death was due to the accumulation of ammonia and that L-arginine was capable of promoting the metabolism of ammonia. It is therefore prudent to consider the question of the mechanism of interactions that have been elucidated from tests with binary mixtures and to assess the importance of the processes of absorption, distribution, and elimination, as well as the major role of interactions with biotransformation pathways.

PHARMACOKINETICS AND TOXICODYNAMICS

When assessing the factors that affect the pharmacokinetics for a single substance, the custom is to reduce a complex biosystem to a relatively simple compartmental model (Gibaldi and Perrier, 1975; Wagner, 1971). This pharmacokinetic model is solely a mathematical concept, which uses well-defined principles to allow the derivation of the simplest equation expressing the variation of concentration of molecular species with time in an accessible body fluid such as the blood, urine, or feces. For a substance such as styrene monomer, which is a lipid-soluble substance distributed to the tissues and extensively metabolized, a single bolus dose administered intravenously in the rat will yield a temporal relationship for the blood concentration (Withey and Collins, 1977). This is adequately described by a bi-exponential equation, characterized by the rate coefficients α and β and the pre-exponential functions, A and B. Clearly, this is a two-compartment pharmacokinetic model for which the hybrid rate coefficients α and β can be obtained either graphically, by the method of residuals, curve stripping, or feathering, or by iterative computer procedures that derive the equation of best fit for the generated data (Mayersohn and Gibaldi, 1971). The derivation of the appropriate pharmacokinetic rate coefficients, k_{12}, k_{21}, and k_e, in terms of the parameters α, β, A, and B from integrated forms of the rate equations for this model, is a relatively simple procedure, which has been well-described in a number of publications (Mayersohn and Gibaldi, 1971; Wagner, 1971).

Since a great many substances are distributed and metabolized by mechanisms similar to those involved in this example, several very important points follow as a consequence of the fundamental principles of pharmacokinetics (Withey, 1976). First, the term biological half-life has little or no relevance when applied to the overall kinetics described by the this model. Biological half-life is the time it takes for the body burden or blood concentration to fall to one-half of its peak value (Snow, 1848; Wagner, 1971) or the half-life of the slowest or terminal elimination phase for the blood-concentration-against-time profile (Levy and Gibaldi, 1975). The half-life concept is very useful for an unambiguous first-order process and is, perhaps, more readily perceived than a first-order rate coefficient and its units or reciprocal time; however, it can cause confusion and errors in the application of well-established pharmacokinetic principles, as in the prediction of steady-state parameters on repeated exposure or dosing (Kruger-Thiemer, 1966; Wagner, 1971).

The pharmacokinetic model rarely relates to, and is seldom synonymous with, the physiological model. Since the latter is of greater interest in evaluating the toxic response of single or multiple insults, the question of the application of kinetic studies to physiological models has been addressed in recent publications (Himmelstein and Lutz, 1979; National Academy of Sciences, 1980).

In many instances where pharmacokinetics has been used to exemplify the nature and extent of altered toxic response, the "rate" (rate coefficient multiplied by the concentration) has been used synonymously with "rate coefficient," and "concentration" has been confused with "amounts." For example, two substances, like the drug tetracycline and the calcium ion, can interact in the contents of the gastrointestinal tract to form an insoluble complex that is not absorbed. Although this interaction reduces the amount of tetracycline absorbed and therefore reduces the overall rate of uptake, it does not affect the rate coefficient of absorption for tetracycline per se. Damage to the gastrointestinal epithelium may alter the mechanism of uptake, and both the rate coefficient and the rate may change; consequently, the amount of uptake will be different.

INTERACTIONS THAT CAN AFFECT ABSORPTION, DISTRIBUTION, METABOLISM, AND ELIMINATION

Many interactions affect the toxicological endpoint, especially in the case of drugs, some of which illustrate specific interaction sites and mechanisms. First considerations cover a number of general aspects.

Any external or internal physiological perturbation might be expected to change the response to single substances and alter the more complex response to multiple insults. The following important factors in this respect have not been considered to any great extent in laboratory models or in carefully controlled studies: environment (heat, cold, stress, vibration, humidity, noise), pre-existing disease (liver, lung, kidney), altered nutrition (diet, malnutrition, food intake, vitamin deficiency, protein deficiency), personal habits (alcohol intake, smoking), rest/strenuous exercise, pregnancy.

Although this paper will not consider each of these in any detail, some statements are significant here: Environmental factors like heat and cold could alter the response to dermal uptake as a consequence of decreased blood flow and other induced physiological changes. A number of studies have demonstrated altered metabolism and physiology as a consequence of extremes of environmental temperature (Baetjer, 1969; Baetjer *et al.*, 1960; Fuhrman, 1946; Inscoe and Axelrod, 1960; Platanow *et al.*, 1963), altered nutrition, food intake, pre-existing disease (particularly that affecting the lung, liver, and kidney), strenuous work, pregnancy, and smoking (Boyd and Boulanger, 1968; Deichman *et al.*, 1972; Dewhirst, 1963; Fuhrman, 1946; Shakman, 1974).

A consideration of the routes of exposure is also of importance since the mechanisms that are involved in the uptake, distribution, and metabolism, and which will ultimately determine the nature and magnitude of the toxic response, may be dependent upon the primary exposure route. Although the three primary routes of exposure (i.e., pulmonary, dermal, and intragastric) should be of principal concern in evaluating the practical situation, in pharmacokinetic and biochemical laboratory models valuable information, such as the nature of dose-response and alterations in the biotransformation pathways and extent, may be obtained from studies after the administration of an intravenous dose.

Indeed, in the consideration of an approach to pharmacokinetic studies, most investigators consider a preliminary examination of the dose-response after intravenous administration a necessary prerequisite to pulmonary or gastrointestinal studies. This is so because the complicating factors known to affect uptake and subsequent entry into the systemic circulation can be avoided (Young and Holson, 1978). For example, the well-known phenomenon of "first pass effect" (Levy and Gibaldi, 1975) can be significant whether metabolic products or intermediates are responsible for the toxicological endpoint or if detoxification pathways are equivalent to the elimination of the toxic moiety. The intravenous route in an animal model surgically prepared with a biliary duct cannula can give immediate indications of enterohepatic recycling; this phenomenon may well play a part in extending the duration of insult regardless of the route of administration.

Biotransformation is really only a part of the four principal aspects of toxicodynamics, but it deserves special consideration. There are numerous examples where the interaction of one chemical substance with metabolizing enzymes can potentiate or inhibit the activity of a second chemical and where well-established biochemical techniques have elucidated the mechanism of action.

Furthermore, the specific nature of the sources and situations of the exposure may be important parameters to evaluate. The workplace, long recognized as a source of multiple exposures to xenobiotic compounds, may well represent the major source of concern for "special groups at risk." A potential for repeated exposure to exotic chemicals, 7 hours per day, 5 days per week, for a working lifetime of up to 50 years, could represent a "worst-case" example. Special consideration of environmental contamination, especially proximate to some industrial activities and dump sites, is also worthy of special attention, as is the contamination of the food chain and potable water.

ABSORPTION, DISTRIBUTION, AND ELIMINATION AS SITES OF INTERACTION

Any factor that alters the rate of absorption, distribution, metabolism, or elimination will have a proportional input on the magnitude of the toxic effect. An empirical investigation into the joint toxic action of selected pairs or more complex mixtures, followed by an investigation of the mechanisms involved, allows not only a more rational quantitative analysis of effects, but can also lend itself to predictions that may be useful in the assessment of a specific multichemical exposure situation. In an attempt to present a concise review of the present state of knowledge, this paper will examine in general the factors governing the rates of mechanisms of the four major pharmacokinetic processes (absorption, distribution, elimination, and metabolism).

Absorption or Uptake

Uptake of a substance into a living biological system requires that it be transported across the epithelium of the lung, skin, gastrointestinal tract, renal tubules, or placenta. This diffusion process involves transport mechanisms that include passive diffusion, usually of neutral molecules down a concentration gradient; active transport, including special interactions and energy; and specialized processes like pinocytosis and ion-pair transport. Once access has been achieved, the systemic circulation transports the substance throughout the organs and tissues of the body. Similar transport mechanisms are involved in the distribution and elimination of the substance. The same consideration must be given to molecular or particle size, lipid solubility, electrostatic charge, or the ability to interact specifically with carrier substrates, for all of these processes.

Most of our knowledge on the uptake of substances by the lung has been derived from an interest in the pulmonary dynamics and properties of general anesthetics. Recently, there has been a growing awareness and recognition of the lung as an important site of toxication, particularly as a consequence of exposures in the workplace (Goldstein *et al*., 1974). Uptake of vapors and gases occurs principally as a consequence of passive diffusion across the epithelial cells of the alveoli in a process usually described by zeroth-order kinetics. Some 300 to 400 million of these alveoli in the human adult form a thin barrier between alveolar air and the richly perfused capillary beds of the blood supply. Molecular characteristics of lipid solubility and size of substances transported by this route are important.

The total surface area of the lung is about 70 m^3 and of the blood capillaries, 90 m^3. The volume of air exchange is between 12 and 15 liters per minute at rest and between 20 and 30 liters at work. In addition, working increases uptake not only as a consequence of the larger volume of air and contaminant drawn into the lungs but also because of an increased blood flow through capillary beds (National Academy of Sciences, 1980). Inhaled solid or liquid droplets of particle size greater than 10 μm are deposited in the nasal passages and pharynx, while particles of about 2 μm or less reach the alveolar sacs. Thus, pollens, aerosols, dust, fumes, tobacco smoke, vapor, and gases with dimensions of less than 2 μm can interact with the alveolar membranes.

The uptake of gas, composed of small lipid-soluble molecules, will usually give a rapidly achieved steady-state blood concentration that is proportional to the exposure concentration, similar to that observed for vinyl chloride monomer (Withey, 1976). A plot of the equilibrium blood concentration against the atmospheric concentration will yield an Ostwald plot for all rapidly acting gaseous anesthetics. Although plots of this kind are useful to assess the extent of uptake from the vapor phase, which will be directly proportional to the slope, in many cases it is the octanol:water partition coefficient that is used in such estimation. For larger, more slowly diffusing, and lipid-soluble molecules like styrene, the time to equilibrium is proportional to the exposure concentration (Withey and Collins, 1979). There may be induced alterations as a consequence of interaction between the inhaled substance and the lung; for example, irritants can alter the pulmonary physiology both in the acute sense and, as in the case of dusts, by inducing lung disease over a longer period of time.

Surprisingly, there are few examples of altered pulmonary uptake due to the inhalation of mixtures (Hayden et al., 1976), although the chronic inhalation of tobacco smoke and other particulate matter from the atmosphere has long been associated with altered pulmonary function and the induction of chronic lung disease (Hammond et al., 1977). Indeed, relevant studies discuss an altered toxic response to mixtures administered by inhalation, and the mechanism usually involves aspects related to altered biotransformation, as opposed to pulmonary transport mechanisms.

After one inhalation of methylene chloride, with and without added ethyl alcohol, the ethyl alcohol antagonized the hepatic damage due to the methylene chloride; but repeated exposure potentiated this respone (Balmer et al., 1976). Two other studies have commented upon the combined effects of gasoline and carbon tetrachloride (Durden and Chipman, 1967) and the simultaneous exposure to benzene and toluene (Forni _et al_., 1971). Both investigations addressed interaction at the target organ or toxicity endpoint. Studies related to exposures of proprietary mixtures have generally been assessed in terms of the action of the most potent toxicant (Gleason _et al_., 1969; Prockop _et al_., 1974).

Different toxicological actions have been observed as a result of repeated exposures when compared with the response to a single exposure, although studies of this kind have been of an empirical rather than rational nature. Repeated exposure to chloroform at three dose levels (85, 50, and 25 ppm), 7 hr per day, 5 days per week for 6 months caused more adverse effects than did a single exposure (Tarkelson _et al_., 1976). Rats, rabbits, and monkeys exposed to 1,2,4-trichlorobenzene at 100, 50, and 20 ppm for 26 weeks revealed liver and renal pathology at 4 and 13 weeks, which was not present, in surviving animals, after 26 weeks of exposure (Coate _et al_., 1977). These results suggest some kind of accommodation or adaptation mechanism not unlike that observed in the case of drugs, such as phenylbutazone, that induce their own metabolism (Herrmann, 1959).

The skin, another absorption site, is structured as a series of membranes coupled with special functions such as sebaceous glands and hair. These membrane layers, from the stratum corneum to the basement membranes, usually act as a barrier to xenobiotics; however, since they are lipid in character, lipid-soluble and small molecules can diffuse across them and pass into the systemic circulation (Goldstein et al., 1974; LaDu et al., 1971). As with the epithelial tissues of lung alveoli, the rates of diffusion across whole skin are roughly proportional to the octanol:water partition coefficient (Treherne, 1956). Of special concern, in terms of multiple or simultaneous exposures, are the low-molecular-weight halogenated aliphatic hydrocarbons, organophosphate, and nicotine insecticides. Although protection of the hand with gloves is advantageous in preventing dermal uptake for many substances, rubber gloves, especially those that are thin-walled, may increase the potential for dermal uptake of small molecules due to the ability of a liquid drop to spread laterally throughout the rubber, thereby increasing the exposed skin surface area.

The skin penetration can be enhanced by dissolving or mixing the compound with a carrier vehicle, such as an oil base or organic solvent. Such is the principle embodied in the majority of topical ointments and emollients employed for pharmaceutical purposes. Dimethyl sulfoxide is a solvent that enhances dermal uptake to an exceptional degree due to its polar chemical structure, combined with its ability to mix with organic solvents (Stoughton and Fritsch, 1964; Weyer, 1967).

The dermal contact response to vinyl chloride monomer, varying from a reddening of the skin to Reynauds' syndrome and even to acroosteolysis, may be a good example of severe reaction to organic substances in the workplace (Harris and Adams, 1967; Lange *et al*., 1974; Viola *et al*., 1971). Other consequences to repeated dermal contact may be hypersensitivity, allergenic response, photoallergic reactions, photosensitization, phototoxicity, and contact dermatitis.

Absorption also occurs along the entire length of the gastrointestinal tract, from the buccal membranes to the colon, and several publications have considered the factors affecting the rate and extent of uptake (Barr, 1968). The gastrointestinal tract has some special properties. First, it is not uniform along its length. Not only does the surface area of epithelial cells per unit length vary enormously but the pH, physical nature, and transit rate of the contents change along its length. Passage into the bloodstream from the gastrointestinal contents necessarily involves transport by the hepatic portal vein directly into the liver. Thus, as opposed to uptake by most other routes, all of the absorbed dose taken up by this route will be immediately subjected to liver metabolism on its first pass. For substances that are rapidly biotransformed to inactive metabolites, the total insult will be less than for a comparable dose administered intravenously or via the lung (Levy and Gibaldi, 1975). Indeed, the drug lidocaine is completely inactivated in a single passage through the liver (Boyes et al., 1971).

Uptake from the buccal cavity is quite efficient, and some pharmaceutical preparations are administered by this route (Goodman and Gilmann, 1972). Pharmacokinetic studies, after administration sublingually or by rinsing the mouth, have been modeled with analogue computers and evaluated in terms of physiological efficiency (Beckett and Triggs, 1967).

Absorption of nutrients and macromolecules from the stomach is negligible. Small, neutral molecules, which include organic acids in the pH 1 to 3 environment of the stomach, are absorbed quite efficiently, and the response to doses of substances like salicylic acid, barbiturates, and aspirin is rapid. Some substances, like insulin, epinephrine, histamine, polysaccharides, and neutral fats, are destroyed by the stomach's acidic environment or the gastrointestinal enzymes and intestinal flora.

Transport of the gastric contents from the stomach to the small intestine is accompanied by a relatively large change in pH. Thus, the pH 7 to 8 of the intestinal lumen allows the facile passage of the neutral forms of acids with a pK_a of greater than 3 and of bases with a pK_a of less than 7.8 (Hogben et al., 1959). The pH of the microenvironment of absorption sites in the small intestine may be very much lower, of the order of 5.3. The very large surface area of the villae, in the 9 meters or so of the human intestine, together with the lipases that catalyze the hydrolysis of the ester linkage of fatty acids and glycerol in triglycerides and phospholipids, makes the small intestine the most important site of uptake for nutrients. Uptake from the distal end of the colon is surprisingly efficient for a great many drug dosage forms. Many irritant drugs are administered by the rectal route to avoid gastrointestinal interaction at the site of lesions, such as ulcers, in the stomach.

Some direct interactions between two substances within the contents of the gastrointestinal tract prevent the uptake of both. Combinations of tetracycline and calcium carbonate form insoluble calcium salts of the antibiotic, leading to poor or negligible uptake (Sweeney _et_ _al_., 1957). Neomycin interferes with the uptake of lipids and lipid-soluble drugs (Gordon _et_ _al_., 1968), and phenobarbital reduces the absorption of griseofulvin (Riegelman _et_ _al_., 1970).

Any substance that interferes with the transit time of the gastrointestinal contents will modify the extent of uptake, although the rate coefficients and mechanisms may remain the same. Thus, osmotic cathartics and diuretics tend to retain osmotic equivalents of water within the tract and inhibit uptake. Analgesics and opiates, like codeine and morphine, as well as anticholinergics, decrease gastrointestinal motility and increase the uptake of substances that are readily absorbed from the stomach. Conversely, cholinergic drugs accelerate the gastric emptying time and depress uptake from the stomach. Other factors like exercise, temperature, food consumption, and emotional status can also affect gastrointestinal motility.

Damage of the gastric mucosa, for example, with tannic acid can increase the rate of uptake of a substance. An alteration in the miscibility and viscosity of the gastrointestinal contents by the coadministration of cathartic doses of a mineral oil can also alter the uptake by a significant amount. The presence of food markedly affects the rate of absorption, not only because of the interaction of an administered chemical with constituents of the food, but also as a consequence of competition with natural food constituents for transport sites, as in the case of methyl dopa competing for the same transport sites of the natural phenolic amino acids.

Distribution

In the majority of instances, the conveyance of an administered dose from the site of uptake to the target organ or site will be controlled by the delivery from the systemic circulation and the partitioning to the tissues, after diffusion through membranes, to the target site. Distribution may, therefore, represent an important aspect for study and give information on the toxicological endpoint. Although the pharmacokinetic model may show that distribution is such a rapid process that it is indistinguishable from the elimination rate (as in a mono-exponential, one-compartment model), the physiological model should identify specifically those sites in the body to which the substance is transported. Variations of pharmacokinetic parameters with dose will usually be indicative of an alteration in distribution or may involve saturable biotransformation pathways.

Storage sites like body fat, as in the case of high-molecular-weight, lipid-soluble substances like some of the pesticides or smaller molecules like styrene, have been called "silent receptors" or "sites of loss" (Levine, 1973). Substances deposited within such sites are released back into the systemic circulation, usually at a very slow rate; nevertheless, they can represent the source of a prolonged insult. Further, any condition that reduces the volume of the storage depot, for example, the case of fat depots during illness or after dieting, can cause more rapid mobilization of the stored compound and, consequently, can induce a severe acute effect.

Sites other than the body fat may be capable of storing substances, and their rapid release could well represent an acute toxic insult. In this respect, substances that bind to blood plasma proteins, like the coumarin anticoagulants, can, after chronic administration, give rise to a situation in which a high proportion of the total body burden is in the bound state. The coadministration of barbiturates, analgesics, antibiotics, and diuretics, which compete for the plasma-protein-binding sites, will result in the displacement of the anticoagulant and may give rise to dangerously high blood levels of the anticoagulant, leading to internal bleeding. The displacement of plasma-bound bilirubin by the administration of sulfonamide drugs or vitamin K can, by the same mechanism of competitive binding, give rise to the potentially lethal condition of kernicterus in the newborn (Goldstein *et al.*, 1974). Many drugs are also bound to tissue sites other than those of the blood plasma proteins. Both pamaquine and quinacrine have been used as antimalarial drugs and are stored in large quantities in the tissues. Quinacrine has a much greater binding affinity for the tissue binding sites, especially in the liver, and will usually displace other drugs or xenobiotics bound or stored at the same sites (Zubrod *et al.*, 1948).

An example of interactions due to environmental contaminants is afforded by the coadministration of polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) to lactating rats (McCormack *et al.*, 1979). Extrahepatic tissue concentrations of PCBs and PBBs were similar regardless of whether they were administered together or alone, although the stimulation of arylhydrocarbon hydroxylase activity was much greater following their simultaneous administration.

Elimination and Excretion

The mechanisms that allow for the elimination of a substance from the blood compartment include those involved in excretion as well as metabolic pathways and distribution, particularly, in the latter case, with respect to transfer to storage sites. Elimination is similar to absorption or uptake but may be considered as the reverse of these processes. Thus, the factors affecting elimination via the membranes adjacent to the feces, the urine, pulmonary air, and the minor routes of passage into the sweat and hair are, in large part, the same for those affecting uptake. This is especially true in the case of the lung.

The kidney is a special excretory organ. Xenobiotics or their metabolites follow principle pathways to pass into the urine and hence to the bladder. Glomerular filtration is a typical filtering process, whereby substances pass through membrane pores at rates proportional to their molecular size and shape. Passive diffusion across the renal tubules, dependent for a large part on similar parameters to those involved in uptake from the gastrointestinal tract, is particularly affected by pH when acids or bases are involved. Many active transport mechanisms, in particular for sugars, amino acids, and organic metabolites, represent the third class of mechanisms available for excretion via the kidney.

Reabsorption via the distal portion of the renal tubules is both an active and a passive process, which can reintroduce active constituents back into the bloodstream in a way similar to that observed in enterohepatic recycling.

At periods of maximal water diuresis, a glomerular filtration rate of 130 milliliters per minute, or about 187 liters per day, are filtered from the plasma. This is about 50 times the volume of the plasma in the body and 15 times the volume of the total extra-cellular fluid. During maximal diuresis, this allows some 114 milliliters per minute to be reabsorbed and 16 milliliters per minute to flow into the bladder (Wagner, 1971).

Interactions that cause severe changes in renal function must include inhibitors of metabolizing enzymes, which would reduce the amounts available for excretion. The enzymes that convert xenobiotics into more water-soluble substances, such as glucuronide conjugates, and the inhibition of such anabolic processes will clearly reduce the elimination of compounds by this active transport pathway (Gillette and Mitchell, 1975). Some aromatic hydrocarbons can actually cause necrosis of the tissues at the site of elimination (Hayden *et al*., 1976) which will reduce the blood volume flow to the organ and thus impair the perfusion of the kidney. Both active and passive renal transport mechanisms are capable of saturation when the administered dose is high enough, which will limit the excretion of some compounds (Gillette and Mitchell, 1975).

INTERACTION INVOLVING BIOTRANSFORMATION REACTIONS

Very few xenobiotic compounds enter and leave the body without being transformed in some way. In many cases the metabolism of these compounds produces an active substance that has a high intrinsic activity compared with the parent compound, whereas in others metabolism will detoxify the parent compound. Any process that interferes or competes with the metabolic pathway of a xenobiotic will therefore modify its apparent toxicity, either to enhance (potentiate) or reduce (antagonize) its activity or prolong its action.

The simplest kind of enzyme interaction with a substrate will result in the reversible formation of an enzyme-substrate complex, which can then decompose to various products. The impact of metabolism on the pharmacokinetics of an administered dose, as monitored by the blood concentration-time course, will be proportional to the extent that metabolism plays a part in the elimination of the compound from the blood. As the size of the administered dose increases and the number of available active sites on the enzyme decreases, so the shape of the semilogarithmic plot for its elimination will become nonlinear, concave to the ordinate axis, as the limiting velocity of the reaction is reached (Gehring *et al*., 1976; Gibaldi and Perrier, 1976). This kind of pharmacokinetic behavior has been demonstrated for a number of xenobiotic compounds such as

2,4,5-trichlorophenoxy acetic acid, dioxane, and vinyl chloride (Sauerhoff _et_ _al_., 1975; Watanabe _et_ _al_., 1976; Young _et_ _al_., 1978).

Although the competitive reversible and irreversible inhibition of an enzyme, such as cholinesterase by phosphonofluoridates and thionates, can precipitate acute and persistent incapacitation or even death, it is the microsomal monoxygenase systems that have attracted the most attention in terms of interactions that induce or antagonize their activity. Recent publications have extensively reviewed this subject (Gelboin, 1971; Remmer, 1969, 1972; Testa and Jenner, 1976).

Almost any lipophilic substance will cause some degree of induction, which is greater for compounds, such as the organochlorine pesticides, that are slowly eliminated from the body. Phenobarbital, amphetamines, and many other drugs cause a general increase, whereas the polycyclic hydrocarbons induce a more specific number; no specific structure-activity relationships have been clearly established.

Although the microsomal oxidases in the principal organ of metabolism, the liver, have been given a great deal of attention, other monoxygenase systems in the extrahepatic tissues of the lung, skin, and intestine may be important where these sites are involved. The enzymes in the latter systems may be different from those in the liver (Alvares, 1977; Grafstrom *et al*., 1977; Wollenberg and Ullrich, 1977). Although extensive literature covers specific compounds that can induce monoxygenase systems, not enough attention has been paid to their mechanism of action. It is usual to give high doses of inducers in laboratory studies, and work is needed in the derivation and evaluation of dose-response data and the investigation of thresholds, if they exist, for these inducers.

A number of conditions can alter induction. Prolonged exposure to the inducing agent is required to induce hepatic response (Remmer, 1969), and though some compounds, like 3-methyl cholanthrene, demonstrate maximum induction within 24 hours, the pesticide chlordane exerts its maximum effect only after several weeks (Testa and Jenner, 1976). Recovery varies in proportion to the rate of elimination from the body. Thus, although complete recovery from the effects of phenobarbital may take only a few days, recovery from more persistent compounds, like the halogenated hydrocarbon pesticides, may take several weeks.

An apparent anomaly exists in the case of some inducers that are known to inhibit metabolism for a short while prior to induction. The well-known mixed-function oxidase inducer, diethyl-aminoethyl-2,2-diphenylvalerate hydrochloride (SKF-525A), and 1,3 benzodioxoles are clearly biphasic in their mode of action. Thus, their initial inhibition over the first 12 hours after administration is followed by a marked stimulation of activity after 24 hours (Testa and Jenner, 1976; Wilkinson, 1976).

A number of in vivo tests for enzyme induction appear to work well. Unfortunately, there is no comprehensive test that applies to all inducers, nor are results in animals always similar in humans. Thus, the steroid metabolites of cortisol appear in the urine so that the ratio of 6 β-hydroxy cortisol to 17-hydroxy cortisol is usually low (Roots et al., 1977). Prior treatment with a number of barbiturates, phenylbutazone, diphenylhydantoin, and a number of other drugs known to induce the monoxygenase system shows a significant and easily detected increase in the excretion of 6 β-hydroxy cortisol. Unfortunately, no increase was observed when animals were pretreated with 3-methyl-cholanthrene-type inducers. This test has, however, shown potential in humans.

Increased excretion of D-glucaric acid has occurred in a number of animal species and humans that were pretreated with drugs known to induce the metabolism of glucose. Many animal species excrete ascorbic acid after the administration of inducers, but humans cannot synthesize this vitamin. It is not usually convenient or desirable to monitor an increase in cytochrome P-450 activity in biopsy samples of human liver, although this mediator is usually a good indicator of induction. Another test for induction, which has shown promise in animal studies, is the measurement of the increase in excretion rate of 4-amino antipyrene, although the toxicity of this and its parent substance precludes its use in humans. There remains a need to develop tests to detect induction, irrespective of the nature of the inducer, which would accommodate the very large intersubject variation in humans that probably arises as a consequence of genetic factors (Vesell, 1977).

The monoxygenase system can, of course, be inhibited competitively and noncompetitively both _in vivo_ and _in vitro_ (Anders, 1971; Mannering, 1971; Testa and Jenner, 1976). The onset of inhibitory effects is usually quite rapid and occurs shortly after administration. Competitive inhibition, sometimes called alternative substrate inhibition, arises from the presence of two oxidizable substrates that compete for the same enzyme site. This phenomenon will be significant only when the combined concentration of the substrates overloads the system. Thus, the most significant effects due to this kind of interaction will occur for those inhibitors that have a high affinity for the enzyme and a low rate of metabolism, like the perfluorinated hydrocarbons that undergo little or no metabolism.

The majority of noncompetitive inhibitors or their metabolites form complexes with cytochrome P-450, which reduces the amount of the latter available for substrate oxidation. Thus, diethyl-aminoethyl-2,2-diphenylvalerate hydrochloride, 1,3-benzodioxoles, and amphetamines inhibit the monoxygenase system in this way (Franklin, 1971; Philpot and Hodgson, 1971; Schenkman et al., 1972). Sulfur-containing compounds, such as thiourea, thioacetamide, carbon bisulfide, and thiobarbital, also interact with cytochrome P-450 due to the release of atomic sulfur, which covalently binds with it (Neal et al., 1977). Direct inhibitors that can covalently bind to P-450 include the imidazoles and other nitrogen-containing heterocyclics (Wilkinson et al., 1974a, 1974b).

A marked induction of serum and liver aliesterase occurs following the administration of drugs and insecticides like aldrin or DDT, which are well-known inducers of hepatic microsomal oxidases (Cohen and Murphy, 1974; Triolo et al., 1970). Aliesterase is an important detoxification enzyme for xenobiotic compounds with carboxyester functional groups, and their inhibition by organophosphates, causing potentiation in the activity of other compounds, has been well studied (Wilkinson, 1976). Epoxide formation from unsaturated linkages in aliphatic hydrocarbon chains or a wide variety of aromatic compounds usually results in the formation of a reactive electrophilic species. Although some of these may be proximal carcinogens, they can also interact with other nucleophiles like glutathione. Thus, the depletion of glutathione or the inhibition of epoxide hydratase would greatly increase the toxic effects of a reactive epoxide.

SUMMARY AND CONCLUSIONS

Pharmacokinetic studies comprise an established and important aspect of the toxicology data base. With the dose administered by the appropriate route, such investigations are most valuable for elucidating the mechanisms of action and understanding or predicting the effects of repeated dosing or prolonged exposure.

Clearly, there must be continued work on the potentiation and antagonism of the enzyme systems involved in biotransformation reactions. Although an empirical approach is still useful for examining the numerous endogenous and exogenous interactions of this kind, an examination of the kinetic principles involved may well lead to a better understanding of structure-activity relationships and their extrapolated role in predicting potential effects.

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Altered Tissue Reactivity and Interactions Between Chemicals

H.P. Witschi[1]

A committee of the National Academy of Sciences (1980) has defined a toxicological interaction as "a circumstance in which exposure to two or more chemicals results in a qualitatively or quantitatively altered biological response relative to that predicted from the actions of a single chemical. The multiple chemical exposures may be simultaneous or sequential in time and the altered response may be greater or smaller in magnitude." Three general mechanisms may underlie these interactions: (a) chemical reactions between two or more of the administered agents; (b) competition of agents for targets, such as the molecular sites for absorption, activation, detoxification, injurious action, or excretion; and (c) alteration of cells by one agent in a manner that profoundly modifies the response to a second agent, even if the first is no longer present.

The first two mechanisms have one feature in common: concomitant exposure or simultaneous presence of the agents is required to affect the biological response. In contrast, the third mechanism does not necessarily require the simultaneous presence of the chemicals. When exposure is sequential, the interaction may be enhancement/inhibition of metabolism or modification of "terrain." Examples of the different types of interactions are shown in Table 1.

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Toxicologists often consider induction or inhibition of mixed function oxidases in various tissues to represent the most important example of the third mechanism of interaction (sequential). Pretreatment with or inadvertent exposure to a variety of agents can profoundly modify the biological response to a second chemical (Conney and Burns, 1972). The toxic manifestations of the second, challenging agent can be enhanced, prevented, or shifted from one target to another. Investigators often interpret interactions of this type in terms of altered metabolic pathways, as in potentiation of haloalkane-induced liver necrosis by ketogenic agents (Hewitt et al., 1980), the shift of 4-ipomeanol toxicity from lung to liver (Boyd, 1980), or protection against chemical carcinogenesis by various dietary regimens (Wattenberg, 1980), among others.

However, not all observations made in studying such interactions are reconcilable by considering only metabolism. Although much evidence suggests that pretreatment with ketogenic agents enhances haloalkane-induced hepatotoxicity by increasing activation of the toxic compounds, some observations

TABLE 1

Toxicological Interactions

Exposure	Type of Interaction	Example
Simultaneous	Chemical-chemical	Oxygen - paraquat Lead - EDTA (ethylenediamine-tetraacetic acid
	Chemical-receptor	SKF525A (diethylaminoethyl-2,2-diphenylvalerate hydrochloride) - drugs Drugs - drugs
Sequential	Enhancement or inhibition of metabolism	Phenobarbital - carbon tetrachloride 3MC (3-methylcholanthrene) - ipomeanol BHA (butylated hydroxyanisole) - carcinogens Carbon tetrachloride - carbon tetrachloride
	Modification of "terrain"	Radiosensitizers Two-stage carcinogenesis Lung fibrosis Isopropanol - carbon tetrachloride

do not fit this hypothesis. At least two investigators have suggested that pretreatment with one compound might produce an increased intrinsic susceptibility of hepatic cells or even subcellular organelles to the second, toxic agent (Davis and Mehendale, 1980; Hewitt et al., 1980), a provocative and interesting hypothesis. Some actual experimental evidence shows that chemicals may increase the susceptibility of cells and tissues to a second toxic agent. Drugs may sensitize tissues, alter cell proliferation, or produce shifts in cell age distribution; such events may alter the biologic response to ionizing radiation (Phillips and Fu, 1978).

Thus, for toxicological interactions where exposure to two agents is not necessarily simultaneous, there are two categories: interactions where unanticipated biological effects are the result of altered metabolism and interactions where cell and tissue responsiveness is modified. For the following discussion, I have selected three examples where exposure to two chemicals produces a qualitatively and quantitatively different biological response and where altered metabolism is not the primary mechanism underlying the toxicological interaction. They are: two-stage carcinogenesis in mouse skin, development of fibrosis in mouse lung, and reduction of bile flow (cholestasis) in rat liver. These examples include enough qualitative and quantitative data to show the complexities of interactions as well as provide definitions of some basic principles.

THREE EXPERIMENTAL SYSTEMS

Two-Stage Carcinogenesis in Mouse Skin

The basic experiment consists in a single application of a known or suspected carcinogen (the "initiator") to the shaved skin of a mouse. One week later treatment begins with application (two to three times a week) of a second agent (the "promoter"). Skin tumors become visible 10 to 12 weeks later. Histologically, most if not all of them are initially benign papillomas, but many carcinomas develop as time progresses.

Parameters to be measured usually include the time to appearance of the first papilloma, the number of papillomas per mouse at 15 weeks, the percentage of mice bearing papillomas at 15 weeks, and the percentage of mice with carcinomas at 50 weeks. In a typical experiment, 100 μmol of dimethylbenzanthracene (DMBA) followed by twice-weekly application of 5 μg of 12-O-tetradecanoylphorbol-13 acetate (TPA) will produce 22 papillomas per mouse after 15 weeks (Slaga *et al.*, 1980).

Development of Fibrotic Changes in Mouse Lung

Mice are injected with 400 mg/kg of the antioxidant butylated hydroxytoluene (BHT) and placed immediately or 1 day later into an atmosphere of 70% oxygen. They are removed from the oxygen 6 days later and kept for another week in room air. Controls are animals injected with BHT and kept in air throughout, mice injected with corn oil and placed for 6 days in 70% oxygen, or mice treated with oil and kept in air. Animals are killed 2 weeks after the initial injection of BHT, and total lung collagen content is estimated by the determination of lung hydroxyproline.

In animals treated with both BHT and oxygen, total lung hydroxyproline usually is 450 to 550 μg per lung, compared to 300 to 340 μg per lung in animals treated with BHT alone, and 200-240 μg per lung in animals treated with corn oil and kept in oxygen or exposed to air only (Haschek and Witschi, 1979). Increased lung hydroxyproline persists up to 1 year after treatment with BHT and oxygen. Morphologically, the inflammatory component of the initial lesion disappears within 2-to-3 weeks, whereas degenerative changes persist. After 1 year, the lungs show distinct areas of emphysematous changes (W.M. Haschek *et al.*, in press).

Manganese-Bilirubin Cholestasis In Rats

Anesthetized rats are kept at 37°C by means of an infrared lamp, and cannulas are inserted into a femoral vein and into the common bile duct. Manganese sulfate ($MnSO_4$) is injected intravenously, and later bilirubin is injected intravenously at predetermined times. Bile collection begins 1 hour later and lasts usually for 60 to 90 minutes. The volume of bile is measured every 15 minutes, and the decrease in bile flow over the collecting period is calculated by taking the volume of the first sample (0-15 min) as the control value (DeLamirande and Plaa, 1978, 1979).

PRINCIPLES OF INTERACTIONS DERIVED FROM THE THREE MODELS

Temporal Sequence Of Exposure

In all three systems, exposure to two chemicals (initiator/promoter; BHT/oxygen; manganese/bilirubin) must occur in the temporal sequence described. If the sequence is reversed, no interactions occur. If mouse skin is treated with a promoting agent first, even for a long period, and then only with an initiator, tumor formation is not enhanced (Slaga et al., 1980). Exposure of mice to oxygen prior to injection of BHT does not produce fibrosis (Haschek and Witschi, 1979). Administration of bilirubin prior to manganese will not reduce bile flow (Klaasen, 1974). Thus, in these examples, the first agent produces a response in the cells of the target organ that will modify the biological response to a second challenging chemical.

These systems show also that the modification of the target by one agent may range from being practically permanent and irreversible to extremely short-lived. In skin carcinogenesis, the initiation step is probably irreversible; promoting agents enhance tumor development even if the interval between the initiating event and the first application of a promoter is extended up to one year or more (Boutwell, 1974). In contrast, excessive accumulation of lung collagen is found if exposure to oxygen occurs immediately or within 48 hours after BHT, but not if it is delayed further (Witschi et al., 1981). Likewise, in rats treated with manganese, bilirubin produces cholestasis only if given within approximately 4 hours, but not if injected later (DeLamirande and Plaa, 1979).

How long tissues exposed to the first agent will remain susceptible to the second chemical depends on the nature of the initial lesion. Initiating agents (carcinogens) are believed to produce permanent changes in the genetic material even after a single application. Administration of BHT, however, produces a reversible lung lesion; it is only during the subsequent few days--the time required to repair the damaged alveolar epithelium--that the tissue is vulnerable to oxygen and that fibrosis will develop. Manganese-bilirubin interactions occur only within a short period--hours rather than days.

Thus, in interactions involving altered tissue responsiveness, the sequence of exposure between the two agents cannot be reversed; the first agent "prepares the terrain" for the second. The interval between the two exposures may be crucial in order to produce a toxicological interaction, and the length of that time interval may vary according to the mechanism of action of the first agent. Of course observation of an ordered temporal sequence of exposure is also important whenever alterations in metabolism are involved. To be effective, inducers of mixed function oxidases need to be given before the second agent, and inhibitors are effective only if given before or at best a few hours after the second agent in order to produce an altered biological response. The two situations--alterations in metabolism and "preparation of the terrain"--thus share one common feature. However, this is not so for the second criterion: the nature of the biological response.

Nature of the Biological Response

There is a fundamental difference between inducers of mixed function oxidases and the three examples. Enzyme inducers usually do not cause a "toxic" response, although they may produce organ changes, such as enlargement of the liver and subtle alterations in cell structure and function, which are often detectable only by electron microscopy or by biochemical measurements. It is the second, challenging agent that is responsible for tissue damage, as in necrosis (Hewitt *et al*., 1980), or whose effect may be mitigated and abolished, as in protection against carcinogenesis (Wattenberg, 1980).

In my three examples, the reverse is true. The nature of the pathologic lesion is determined by the first agent, whereas the second agent allows or enhances expression of the lesion. Many carcinogens, including DMBA, produce skin tumors without promotion; BHT alone has some fibrogenic potential in mouse lung; and manganese can produce cholestasis on its own. On the other hand, the agents used to enhance substantially the development of these lesions are devoid of any such activity. Repetitive application of TPA on mouse skin without an initiating agent does not, in general, produce tumors; when they do appear, it is not in a dose-dependent manner (Slaga *et al*., 1980). In experiments designed to promote liver tumor formation by phenobarbital, dichlorodiphenyltrichloroethane (DDT), or BHT (Peraino *et al*., 1975, 1977) or to promote lung tumors by BHT (Witschi and Lock, 1979), the enhancing agent alone does not increase the tumor incidence or multiplicity. Oxygen in concentrations used to produce lung fibrosis in BHT-damaged lungs (70% for 6 days) does not produce a measurable accumulation of lung collagen, and bilirubin given alone is not cholestatic. In all three examples, therefore, the first agent dictates the qualitative nature of the adverse response; the second agent accelerates or enhances this development, but does not produce the same response if given per se.

This statement needs some qualification. Some investigators consider TPA to be a complete carcinogen (Iversen and Iversen, 1979); others have found oxygen alone to produce lung fibrosis in rats (Valimaki _et al_., 1975); and bilirubin causes cholestasis in monkeys (Gartner _et al_., 1971). However, these results occurred in differently designed experiments, with different doses and different animal species or strains. The basic principle stands that, in interactions involving metabolism, the biological response is determined by the second, challenging agent, whereas, in interactions involving altered cell and tissue responsiveness, the second agent aggravates a response ordinarily produced by the first agent.

Dose-Effect Relationships

In two-stage carcinogenesis, lung fibrosis, and cholestasis, the qualitative nature of the biological response seems to be determined by the first agent. The extent of the response, however, is dictated by both the first and the second agent. In mice treated with various amounts of two carcinogens--benzo(a)pyrene and DMBA-- and given a fixed amount of promoting agent (5 μg of TPA twice weekly for 15 weeks), the number of papillomas found at 15 weeks is proportional to the dose of the initiator. If the dose of the initiator is held constant and the amount of promoter varied, there is a correlation between number of tumors per mouse and total dose of promoting agent applied (Slaga _et al._, 1980).

Interactions between BHT and oxygen have produced similar results. If animals are given various doses of BHT and placed for 6 days into 70% oxygen, the amount of excess lung hydroxyproline increases with larger doses of initially given BHT. If animals are treated with a constant dose of BHT and placed for 6 days in an atmosphere of oxygen ranging from 40% to 80%, excess lung hydroxyproline is proportional to the oxygen concentration. Doses of BHT or oxygen concentrations alone are, however, not the only determining factors. BHT treatment (100 to 400 mg/kg) followed by 6 days of exposure to 70% oxygen will significantly increase lung hydroxyproline at all BHT doses. But, if oxygen exposure is reduced to 3 days, fibrosis develops only in the group given the largest dose of BHT (400 mg/kg) and not in animals treated with smaller doses (Witschi _et al._, 1981).

In manganese-bilirubin-induced cholestasis, dose-effect and time-effect relationships are equally complex (DeLamirande and Plaa, 1979). Injection of 4.5 mg/kg of manganese 15 minutes before 10 to 35 mg/kg bilirubin reduces bile flow in a manner similar to that resulting from 6 mg/kg of manganese injected 30 minutes before bilirubin. On the other hand, the dose of injected manganese determined the time interval up to which administration of a fixed dose of bilirubin reduces bile flow by 50%. After 4.5 mg/kg of manganese, bile flow following bilirubin is only reduced by 50% or more if the bilirubin is injected not more than 30 minutes later. If 9 mg/kg of manganese is given, bile flow is still reduced if bilirubin is given almost 4 hours later (DeLamirande and Plaa, 1979). Therefore, the dose of manganese determines the period of time during which manganese-bilirubin cholestasis can be produced. The larger the dose of manganese, the longer the time interval can be between its injection and that of bilirubin in order to reduce bile flow.

Certainly, a systematic exploration of dose-effect and time-effect relationships is extremely complex. Possible variables include dose of the first agent, dose of the second agent, time interval between administration of the two agents, and duration of exposure to the second agent. An analysis of the presumably equal complexity of potential dose-effect and time-effect relationships in situations where metabolic activation of the challenging second agent is the key element goes beyond the scope of this paper.

Expression of Hidden Responses

In these types of interactions, the first agent determines the qualitative nature of the biologic response. Given the appropriate conditions, that response is expressed only if an interaction occurs. The classical example is two-stage carcinogenesis. Application of a promoting agent will result in the development of tumors even if the initiator was given at a dose too low to produce tumors alone (Slaga _et_ _al_., 1980); only through the interaction of initiator and promoter is the particular biologic response expressed. In the BHT-oxygen model, doses of 200 mg/kg or less of BHT do not produce a significantly higher amount of lung hydroxyproline; only a subsequent exposure to oxygen leads to fibrotic lung changes (Witschi _et_ _al_., 1981).

Even more instructive is some recent work on ultraviolet (UV) radiation carcinogenesis in mouse skin (Fry _et_ _al_., 1982). In one experiment, exposure of two strains of mice to a controlled dose of PUVA (i.e., UV-A plus 8-methoxypsoralen) produced the same number of psoralen-DNA (deoxyribonucleic acid) crosslinks in the epidermis of both strains. Thus, the initiating dose was equal in both strains. However, 80 weeks after the experiment began, only 5% of all mice in one strain had tumors, whereas the other strain had a tumor incidence of approximately 60%. When the animals were treated with the tumor-promoting agent TPA, tumor incidence at 80 weeks was about 85% in both strains. The apparent difference in susceptibility to a carcinogen between the two strains was associated with differences of expression of the initial event, and expression was enhanced by a second agent. Thus, toxicological interactions may influence the development of a lesion that would have been much more suppressed in one strain than in the other. However, once expression was maximized, the apparent strain difference disappeared.

Furthermore, Fry *et al*. (1982) also observed that the dose-response curve resulting from exposure to PUVA is curvilinear and shows a definite threshold; below a certain dose, no tumors develop. However, if the exposure to PUVA is followed by a promoting treatment, the dose-response curves shift to the left and show a no-threshold linear response. Thus, toxicological interactions may (a) unmask lesions that otherwise would have gone undetected, (b) allow the expression of lesions, and (c) by doing so, change the shape and the position of the dose-response curve.

DISCUSSION OF THE THREE MODELS

The three examples describe experimental models where exposure to a second agent allows expression or amplification of the biological response elicited by a first agent. They also are animal models of human pathologic conditions: cancer, chronic lung disease, and cholestasis. For these to be truly representative models, enhancement of tumor formation, development of lung fibrosis, and potentiation of cholestasis must occur in other experiments that are different from the ones described so far.

Ten years ago scientists were not certain whether the principles of two-stage carcinogenesis, as developed in mouse skin, would apply to other epithelial tissues, particularly of internal organs. Researchers have since observed tumor promotion in internal organs such as liver, lung, colon, urinary bladder, and esophagus (Slaga _et al_., 1978). In each case, they found they could enhance tumor formation, once initiated by a carcinogen, by subsequent administration of compounds that are weakly or not at all carcinogenic per se. Moreover, they determined that many agents other than TPA could act as promoters in mouse skin (Slaga _et al_., 1981). The difference between various agents lies in their potency--that is, the amount that must be applied to effect promotion--and not in their inherent capability to produce more tumors. This obviously broadens the implications of work done with TPA and allows extrapolation of mechanistic and quantitative observations to other promoters.

The principles underlying two-stage carcinogenesis in mouse skin are thus generally applicable. Carcinogenesis is a multistage process, and the events associated with tumor expression are probably as important as events leading to initiation. Moreover, tumor expression may be profoundly modified by interactions with agents that have only limited, if any, carcinogenic potential per se. It is thus an important development that there are now methods for identification of promoters in _in vitro_ assays that measure cell-to-cell communication (Yotti _et al_., 1979). Mechanism studies of these kinds will not only advance our basic understanding of promotion in animals and humans, but also might yield a rational approach to identifying promoters.

The experiments conducted with BHT have shown that a damaged lung is much more susceptible to oxygen and possibly to other toxic inhalants as well. The interaction of BHT and oxygen cause an essentially irreversible lesion to develop, but does oxygen exposure produce fibrosis in other models of acute lung injury? So far we have found that fibrosis develops if animals with lung damage produced by bleomycin, cyclophosphamide, methyl cyclopentadienyl manganese tricarbonyl, or inhaled cadmium chloride are exposed to oxygen (Hakkinen *et al*., unpublished observations, and Witschi *et al*., 1981). Under these circumstances, fibrosis may develop because the first agent damages the lung and, during the recovery phase, a second cytotoxic agent damages the proliferating epithelial cells. Experiments to verify this hypothesis revealed that, when dividing epithelial cells were killed by x-rays instead of by oxygen, extensive lung fibrosis developed and persisted up to 6 months (Haschek *et al*., 1980; Witschi *et al*., 1980).

In summary, the principles underlying BHT-oxygen-induced fibrosis in mouse lung apply to several other examples of toxicological interactions in mouse lung and are not limited to the mechanism of action of BHT or of oxygen. The general principle is, therefore, that a damaged lung is much more susceptible to a toxic inhalant than is a normal lung. An essentially irreversible chronic lesion develops (fibrosis and emphysema) if a second toxic agent compromises the phase of tissue recovery in a damaged lung. This may apply to human lung disease such as adult respiratory distress syndrome (Pratt _et al_., 1979) or accelerated development of fibrosis following concomitant administration of anticancer drugs and therapeutic irradiation of the thorax (Gross, 1977). Experiments should continue on other organs (e.g., liver, kidney) to determine if a damaged tissue is more susceptible to another toxic agent and if further exposure to that agent will develop chronic degenerative lesions.

Throughout this discussion, I have interpreted the studies on managanese-bilirubin interaction as an example of a toxicological interaction most likely not caused by alterations in metabolism. This interpretation has not been ruled out completely. Potentiation can be explained by an enhancement of biotransformation leading to the production of toxic metabolites or by an increased susceptibility of the subcellular organelles of the hepatocyte (DeLamirande and Plaa, 1978, 1979). However, more recent work on enhancement of cholestasis by 1,3-butanediol pretreatment in rats given manganese-bilirubin, taurolithocholic acid, or α-naphthylisothiocyanate favors the view that the basic mechanism involved is indeed increased cell susceptibility (DeLamirande and Plaa, 1981).

Drug-induced cholestasis occurs in humans, but attempts to observe this by treating experimental animals with the drugs have met with little success, if any (Plaa and

Priestley, 1977). Development of cholestasis may involve an allergenic (hypersensitivity) reaction, or cholestasis in humans may be a result of interactions of two agents. The comparatively rare incidence of the disease might be due to the infrequent occurrence of the right conditions necessary to provoke a response (critical dose and time-relationship between the two interacting agents).

GENERALIZATIONS FOR MULTICHEMICAL EXPOSURES

The three documented examples of toxicological interactions cannot be explained solely in terms of enhanced or inhibited metabolism of one agent by another. They are examples of altered cell or tissue responsiveness. If we accept this to be an important phenomenon in the pathogenesis of acute and chronic tissue lesions, we may define the following general principles:

1. An important factor is the temporal sequence of exposure to two agents. Unless exposure occurs within a given time interval, no interactions occur. The requisite time interval depends upon the mechanism of action of the first agent. However, if the sequence of exposure is reversed, no interactions occur, regardless of the timing of exposure.

2. The qualitative nature of the biological response is determined by the first agent, whereas the quantitative response may be modulated by both the first and the second agent. Time-effect and dose-effect relationships are complex.

3. Interactions between two chemicals may allow the expression of lesions that otherwise would not develop.

4. In cases where one agent alters tissue or cell reactivity so as to modify the response to a second agent, the key element is the nature of the biological response, not the initial mechanism. BHT probably causes lung damage by a different mechanism than does cadmium chloride, yet both compounds produce cell death in the lung followed by cell proliferation. It is the interference with a secondary event, tissue recovery, by oxygen or by x-rays that then leads to abnormal accumulation of collagen. Manganese, taurolithocholic acid, and α-naphthylisothiocyanate have different initial mechanisms of action, but all three produce a stasis in bile flow (DeLamirande and Plaa, 1981). Predictions of interactions must thus be made by knowing the dynamics of the biological response, rather than by considering chemical or metabolic concerns that apply to chemicals and not to biology.

An analysis of the interaction of drug and radiation effects on normal tissue has recognized similar basic principles (Phillips and Fu, 1978): The tissue expressing the injury is critical and related to the drug's specific toxicity; drug dose and time of administration in relation to radiation exposure are important, as are the cell kinetics of the target tissue.

CONCLUSIONS AND RECOMMENDATIONS

The interactions I have discussed were not discovered by blindly testing mixtures of chemicals, but by studying biological phenomena: skin tumors in mice, acute pulmonary injury, and intrahepatic cholestasis. At a particular time each experiment addressed the question of how the initial biological response might be modified. There was always a rationale available, which in turn led to the design of appropriate experiments. Croton oil was initially used to produce skin irritation as a means to reexamine critically and in a controlled experiment whether chronic irritation played a role in carcinogenesis (Berenblum, 1944). Studies with BHT suggested that proliferation of lung epithelial cells might be adversely affected by oxygen, whereas fibroblasts seemed to be resistant; killing epithelial cells would allow an overgrowth of fibroblasts (Witschi and Cote, 1977). Overloading rats with manganese produced a condition that suggested intrahepatic cholestasis, and bilirubin infusion provided evidence for inhibition of bile transport (Witzleben _et_ _al_., 1968).

Toxicological interactions of this type are thus most likely discovered through rational, although often unconventional, experiments, specifically designed to answer thoughtful questions. In each system, the biology was initially (and still is) better understood than the molecular mechanisms involved. The discovery of interactions through an all-encompassing testing scheme, not based on biology, may be elusive unless we can devise a scheme that will anticipate all possible modifications of all possible biological responses, clearly a near-impossibility. Moreover, the importance of temporal relationships all but precludes the hope of finding certain types of interactions by exposing animals concomitantly to mixtures.

Once there is evidence of an interaction, investigators must thoroughly study the dose-effect and time-effect relationships. This calls for experimentation with a great number of animals under a variety of exposure conditions. The number of variables to be tested can be considerable. Information is obtained efficiently only if end points to be measured are simple, quantitative, reproducible, and easily acquired. Counting tumors, though obviously a very simple procedure, has provided the most information in two-stage carcinogenesis. Measurement of total lung hydroxyproline or of bile flow are also comparatively simple procedures.

In other words, once we have defined the response to toxicological interaction, we must evaluate it with the simplest suitable technique to obtain the necessary amount of data with a reasonable effort. We may choose mechanistic studies involving more sophisticated approaches only after we have defined the optimum and most relevant conditions to produce the desired effect. To begin an analysis of the biological phenomenon with the most advanced and involved techniques may initially be a waste of time and resources. Comparatively crude end points, selected with careful consideration of what they mean, will yield more meaningful answers than the most involved biochemistry, physiology, or morphology. Interactions must be anticipated, analyzed, and interpreted from the biological response they produce.

Two-stage carcinogenesis in mouse skin, BHT-oxygen-induced lung fibrosis, and manganese-bilirubin cholestasis are attractive models for the study of some interactions. Models are, however, insufficient unless we ascertain that they truly represent a pathogenetic principle. We may learn more about a steam engine by looking at a boiling teapot than by playing with an electrically driven toy engine, although the latter may superficially look much more like the real thing than does a teapot, and is also more fun to play with. When we believe we have a model, we must design experiments to broaden its implication for biology. We put the principles we deem important into different experimental systems where they might apply and then determine if we still get the same basic biologic response. Two-stage carcinogenesis is a good model because we have experimental as well as speculative evidence that it applies to epithelial tissues other than mouse skin. The proposed pathogenesis of lung fibrosis in mice will be a better model when we can show that its mechanistic principles apply to species other than the mouse and perhaps to other tissues. Only then may we proceed to analyze the model system in greater detail. We should not focus too early or even exclusively on molecular mechanisms in biological systems and neglect the behavior of cells and tissues.

Finally, we need to learn where to suspect toxicological interactions. We should watch for unusual or unexpected biological responses in both humans and animals, which may alert us to the possibility of toxicological interactions.

Examples are the discovery of chlordane as a powerful inducer of mixed function oxidases (Fouts, 1963) or the "Couch Case," in which the usually brilliant Dr. Alexander overlooked the potentiation of carbon tetrachloride hepatotoxicity by ethanol, although it can readily be deduced from the circumstances surrounding the accidental death (Celwyddwr, 1974). We may also consider that diseases of unknown, cryptogenic, idiosyncratic, or idiopathic etiology may occasionally be caused by multichemical contamination, under certain conditions of exposure with regard to both dose and time.

The toxicology of interaction is the toxicology of two or more variables. Such complexity necessitates an attempt to recognize, understand, formulate, and test the principles underlying the biological response, as well as those concerning its modification, at all levels of biological organization.

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Studies of Human Populations Exposed to Environmental Chemicals: Considerations of Love Canal

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This paper discusses studies of persons exposed to environmental chemicals, the limitations of these studies, and future approaches to dealing more effectively with this complex issue. In the most rudimentary way, such come to our attention through reports of illness, reports of exposure, or some combination of both. A report of illness results in an evaluation of exposure, whereas the report of exposure generates an evaluation of the health of the exposed individuals. Depending on the number of people exposed, the type and degree of exposure, and the nature of any illness involved, a designed epidemiologic study may be indicated. Thus, these investigations can take any of three general forms: crisis response, evaluation of the health of individuals, or designed epidemiologic studies.

Response to illness or crisis response is necessary when episodes result in death or sudden acute illness in small groups of the population. The incidence of illness must be in excess of naturally occurring background disease (Abercrombie, 1953; Cam, 1963; Diggory et al., 1977; Firket, 1931; Kuratsune

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et al., 1969; Pierce et al., 1972; Shrenk et al., 1949). The cause of the outbreak must be established so that appropriate treatment and control measures can be provided.

More commonly, situations arise in which members of a population are concerned that their health may have been affected by exposure to a particular chemical. Unless exposure has been well-established by measuring levels of chemicals in environmental media or in tissues, such evaluations are unrewarding and should not be undertaken especially if their primary goal is to alleviate fear. If it can be determined that exposure was minimal and could not possibly lead to recognizable health effects, then no health evaluation should be done. It is unethical and wasteful to subject persons to extensive health evaluations and laboratory tests if it is known that the outcome will be negative or that whatever findings are made cannot be related to exposure.

If little is known about the toxic effects of the chemical or if the degree of exposure is not well defined, limited health evaluations may have to be conducted. These health evaluations should be well focused and should concentrate on the most likely effects that the chemical might produce. Health evaluations should not be "fishing expeditions," i.e., conducted with little thought about their meaning or usefulness.

This paper deals with the third category: epidemiologic studies based on study protocols with clearly defined end points, such as adverse reproductive outcomes, incidence of cancer, and frequency of chromosomal aberrations. Before initiating such studies, investigators should establish the size of the exposed population and the nature and degree of exposure. Similar information on a comparison group is usually required. They should determine the baseline estimate of the incidence of the disease outcomes in question so that they can determine whether studies are feasible and have a reasonable chance of documenting those health effects.

CONDUCTING EPIDEMIOLOGICAL STUDIES

Experimental studies conducted by deliberately exposing a human population to chemicals are warranted only if exposure is deemed beneficial, such as fluoridating community water supplies to reduce tooth decay (Blayney and Hill, 1967). With the exception of drug trials, most human exposures occur accidentally (Carter 1976; Carter *et al*., 1975; Kreiss *et al*., 1981a, 1981b; Landrigan, 1979; Vianna, 1980) and are uncontrolled. On the next page is an outline with examples of health effects that could be investigated. The types of endpoints that might be investigated depend on the chemicals to which people were exposed.

In many instances, information about the toxic effects of chemical compounds in humans is scanty or nonexistent. Even less information is available on effects produced by a combination of chemicals. Although different species may respond differently to the chemicals, results obtained in animal studies can be used to decide what effects should be

OUTLINE OF ASSESSMENT OF HEALTH EFFECTS IN HUMAN POPULATIONS AFTER CHEMICAL EXPOSURES

I. Kinds of measurements
  A. Exposures
    1. Agents
    2. Metabolites
  B. Outcomes
    1. Symptoms (rashes, eye irritation)
    2. Signs (rashes, paralysis, tremor, etc.)
    3. Disease or disorder
      a) Apparent
        (1) Abnormal reproductive outcomes
        (2) Growth and developmental disorders
        (3) Behavioral or psychological disorders
        (4) Cancer
        (5) Other disorders (autoimmune diseases, blood dyscrasias, coronary artery disease)
      b) Inapparent
        (1) Biochemical abnormalities (cholinesterase, erythrocyte protoporphyrin, liver function tests)
        (2) Immunologic abnormalities (lymphocyte tests)
        (3) Chromosomal abnormalities
        (4) Nerve conduction abnormalities
        (5) Other test abnormalities (pulmonary function)
  C. Other factors mediating, confounding, or interacting with exposures and outcomes (age, nutrition, migration, occupation)

II. Methods to assess relationships between exposures and outcomes
  A. Kinds of studies
    1. Experimental (control of exposure)
    2. Nonexperimental (no control of exposure)
      a) With concurrent comparison groups
        (1) Cohort (Cross-sectional and/or longitudinal)
        (2) Case-control (cross-sectional and/or longitudinal)
      b) Without concurrent comparison groups
        (1) Formal surveillance
          (a) Exposed populations
          (b) Populations with specific outcomes
        (2) Case clusters
        (3) Anecdotal reports
  B. Quality control methods
  C. Statistical methods

considered when studying accidentally exposed human populations. A toxic effect may, of course, be demonstrated first in humans (Folland et al., 1978). A suspicion that illness was caused by a certain chemical should not be disregarded even if the effect has not been reported in animals. On the contrary, attempts should be made to develop an animal model in which the disease can be produced.

Although experience gained in animal studies can aid in designing human studies, there are some difficulties and limitations in this approach. In animal studies, researchers control the number of animals, the dose, age, sex, nutrition, and environment; they know the incidence of background disease and the genetic makeup. None of these factors is established when uncontrolled exposure has been or is occurring in humans. In such situations, the epidemiologist has two types of approaches: cohort studies and case-control studies. Cohort studies mimic experimental studies by following groups exposed and not exposed to an agent (e.g., polybrominated biphenyls in Michigan) and determining whether the effect (e.g., illness) occurs more often among the exposed. Case-control studies compare those who have experienced the effect (the "ill" cases) with those who have not (the "well" controls) to determine whether exposure has occurred more often among the cases. Cohort and case-control studies may be thought of as cross-sectional or longitudinal in nature. Cross-sectional studies measure exposure and illness at the same point in time

(Kreiss et al., 1981a, 1981b); longitudinal studies relate these measurements to two different points in time. Combinations of these studies are also possible (e.g., doing a case-control study among incident cases occurring during a cohort study follow-up).

Feasibility

Before undertaking such studies, investigators must determine if they are feasible. If very few people were exposed, a study may not yield meaningful results. For example, in 1971 several riding arenas in Missouri were sprayed with salvage oil contaminated with 2,3,7,8-tetrachlorodibenzodioxin, polychlorinated biphenyls, and 2,4,5-trichlorophenol (Carter et al., 1975). Although many animals became ill or died, fewer than 10 persons had more than casual exposure to the contaminated arenas. The health of the individuals in this group has been evaluated repeatedly, but, because of the small number of persons and their heterogeneity (differences in age and sex), there has been no epidemiologic study of this group.

Occasionally, if specific outcomes, such as cancer, are suspected, individuals may have to be closely followed. This would be the case in workers who had been exposed to known bladder carcinogens or in a recent episode where several individuals ingested a toxic dose of dimethylnitrosamine (Cooper and Kimbrough, 1980). The aim of such efforts is to detect cancers as early as possible--that is, while they can still be treated--rather than to demonstrate effects in the framework of a study.

If large numbers of people have been exposed to a chemical or a mixture of chemicals, a designed epidemiologic study may be feasible. For example, there was an accidental exposure of a sizable number of the Michigan population to polybrominated biphenyls (PBBs) when they consumed PBB-contaminated milk, eggs, and meat (Landrigan *et al*., 1979). A similar situation exists in Triana, Alabama, where the population of a small town (several hundred people) for many years ate locally caught fish contaminated with PCBs and high concentrations of dichlorodiphenyltrichloroethane (DDT) and related materials (Kreiss *et al*., 1981a, 1981b).

Limitations

Closer examination of these groups, however, shows additional limitations inherent in these studies. In Michigan, the total amounts of PBBs to which individuals were exposed varied a great deal, resulting in a wide range of PBB body burdens. Furthermore, PBBs are a mixture of compounds, some of which may be more toxic than others (Patterson *et al*., 1981; Robertson *et al*., 1981). Any measurement of total PBB body burden may not reflect differences in exposure to specific PBB isomers. Furthermore, the Michigan population had also been exposed to polychlorinated biphenyls (PCBs), which may potentiate the toxic effects of PBBs. The exposure to PCBs was not uniform in this population.

Since PBBs, PCBs, and DDT residues are very persistent, body burdens do not change rapidly. Therefore, we can establish exposure levels in these populations and use them as points of reference. Since the general population is very heterogeneous, some individuals with lower body burdens may have symptoms, but others with higher body burdens may not (Cannon _et al_., 1978).

Although investigators in the PBB study have established a cohort of 4,000 exposed people, it still may not be possible to demonstrate adverse effects of health. There was varied exposure, with a mean PBB blood level in the cohort of 21.2 μg/liter of serum and a range of 0 to 1,900 μg/liter of serum; age at the time of exposure varied a great deal; and both males and females were exposed. A similar situation exists in Triana, where exposure occurred over a longer period than in Michigan, but the cohort is much smaller. On the other hand, PBBs are much more persistent than chlorinated aromatic compounds. Fortunately, in both situations, the population seems relatively stable; it is not greatly affected by migration; and it is very cooperative.

Biases

In these studies, certain biases must be avoided (Sackett, 1979). A bias may occur when the knowledge of an exposure influences the diagnosis of or treatment for an illness. In cohort studies, those in the exposed group who become ill may be diagnosed and treated earlier than those in the control group (selection bias). Continued follow-up or periodic and comparable examinations of both the exposed groups and the control group can reduce this bias. Another bias may occur when the definitions of exposure and illness are inadequate or when the information obtained from the exposed group differs from that obtained from the control group (misclassification bias). A third bias may be introduced when a factor may be involved in part, but not all, of the relationship between an exposure and an illness (confounding bias). This might occur in a study of the environmental exposure to a chemical when a member of the exposed group also had been occupationally exposed to a chemical causing a similar effect. In that case, the study should be designed to eliminate this effect, or enough information should be collected to make it possible to adjust the analysis for the effect.

CONSIDERATIONS OF LOVE CANAL

An entirely different situation from those in Michigan and Triana existed in Love Canal, a chemical dump in Niagara Falls, New York. From 1947 to 1952, Hooker Chemical Company dumped more than 20,000 metric tons of chemical wastes into the canal. In 1953, the company closed the landfill and sold the property to the local Board of Education, which developed the area for residential use. Mostly one-family homes were built on both sides of Love Canal, with a public elementary school adjoining the central section. In 1978, local residents became concerned that chemicals were leaching from the canal into adjacent soil. Intensive sampling of air, soil, and groundwater by the New York State Department of Health and the U. S. Environmental Protection Agency (EPA) led to the identification of chemicals in the basements of several homes adjacent to Love Canal. Eventually, more than 200 chemicals were identified throughout the Love Canal area.

In the fall of 1978, New York State authorities began relocating the residents and the state purchased the homes of the 239 families residing in the first two rings of homes around the canal. Furthermore, it conducted geological surveys, to gain a better understanding of possible modes of human exposures, and ran an extensive interview survey concerning possible human health effects. The latter study produced data suggesting possible reproductive effects in families living either adjacent to the canal or in homes built along historically wet areas, where water seepage may have been a particular problem (Vianna, 1980). To date, no other scientifically based evidence suggesting human health effects has been advanced, although many anecdotal reports of illness have circulated in the community.

After the state bought the two rings of houses adjacent to Love Canal, state workers dug a trench around the canal. They installed drains in the trench to remove leached chemicals, and they capped the dump with a thick clay cover. A waste treatment plant on the site now decontaminates all leached substances collected in the drains around the canal.

Public concern about the potential health hazards from chemical exposure and about the economic impact on property values continued to grow, despite progress in containing the dump site and efforts to address community needs. Work by other groups, such as the Environmental Defense Fund, has included studies of child development, nerve conduction velocity (preliminary testing inconclusive), and cytogenetic effects. The last study, initiated in January 1980 under EPA auspices and reported in May 1980, showed chromosome damage in 11 of 36 persons tested for chromosome breakage (Kolata, 1980). This report stirred much scientific controversy; outside review groups raised serious questions about the study's methodology and the interpretation of its results in the face of apparently inadequate control material. Nonetheless, the report was partly responsible for the U. S. government's designation of the Love Canal neighborhood as a disaster area and its relocation of several hundred more families from the area. Extensive efforts were made to design additional comprehensive health effects studies; however, none has been started.

Even if studies were conducted, the outcomes would be, at best, difficult to interpret. Some of the people who lived or are still living near Love Canal are convinced that their health has been affected. Disenchantment with past studies and distrust of the government at all levels might result in poor participation in such studies. Selection bias from such limited participation would severely limit any conclusions.

Most of the chemicals in the dump site that the population around Love Canal could have been exposed to are rapidly metabolized and excreted. Environmental levels of chemicals were not well characterized before the canal was capped. Therefore, no precise exposure data are available for the population living in the area. If effects are reversible, those who were moved in 1978 would no longer yield positive findings or have detectable residual chemicals in their bodies. They were probably the group at highest risk. The lengths of exposure times and the chemicals in the dump to which particular persons were exposed are also unknown. All people living around Love Canal may not have been exposed, and most who were exposed were only exposed to very low chemical concentrations, much less than those encountered in some occupational settings. Whether the combination of many chemicals, even at low concentrations, would have adverse effects on human health is not known.

Although TCDD (2,3,7,8-tetrachlorodibenzodioxin) in μg/kg concentrations has been found at Love Canal in samples from the dump and in the surrounding storm sewer system, actual human exposure to it has not been demonstrated. Thus, exposure to more persistent chemicals, as in the Michigan or Triana situations, cannot be documented at Love Canal. EPA measurement of serum levels of chemicals in 36 people did not show any evidence of exceptional exposure. Against this background, statistical and epidemiologic assessments for potential studies were made. (See the outline on page 292 for the types of studies or health evaluations that may be useful in exposure situations like these.)

Many effects caused by chemicals also occur naturally in the general population. Depending on the natural background incidence of disease, demonstrating a doubling of an effect may require a study population of anywhere from 100 to several thousand people. For example, to detect an increase in illness prevalence from 0.1% to 0.2% with 80% statistical power at a one-sided significance level of 5% would necessitate 20,400 unexposed and 20,400 exposed people. This small increase in prevalence may not seem significant, but extrapolating it to the entire population of the United States (226 million) would affect 226,000 additional people.

A further problem is baseline data in the general population. Even for such simple parameters as liver function tests, no good baseline information is available. Many of the techniques listed in the outline have severe limitations in terms of methodology and normal values. More sophisticated tests, such as chromosome studies, are not yet part of the mainstream of medical practice. Extensive baseline data are lacking, and disagreement and confusion exists among scientists working in this area. The Centers for Disease Control, in collaboration with EPA, other federal agencies, and the scientific community, are developing guidelines in an attempt to promote more orderly studies and to point out pitfalls that may lead to erroneous results or misinterpretation of data (Bloom, 1981).

Among the pitfalls are such mundane factors as the accidental contamination of or improper collection of specimens in the field. Specimens may be improperly preserved or transported. Unless we have well-standardized, analytical methods, results may not be reproducible. If measurements such as liver function tests are done in a population over time, they must always be performed in the same way, and a quality control system must exist to detect drifts in methodology. Such a quality control system has been instituted for PBB determinations in the long-term follow-up study in Michigan (Burse *et al*., 1980).

In addition to our lack of knowledge about baseline data in the general population in many areas listed in the outline, we also lack knowledge about how some of these effects or differences relate to an individual's personal health. In some cases, it may be unethical to conduct tests in humans when the significance of the results, and how those results relate to health or future illness, cannot be properly interpreted for the individual. Because of these difficulties, lack of evidence of an association between an exposure and a health effect is, in most cases, not evidence for lack of an association. On the other hand, because of bias or chance, a positive association may be erroneously noted.

Thus far, we have discussed relationship between exposure and health effects without much attention to exposure to several chemicals. When more than one exposure is involved, the existence of distinct relationships between each exposure and the illness must be verified, but the relationships among the exposures must also be examined. One exposure may confound another, explaining part or all of the relationship between the exposures and the illness. The first exposure may interact with or modify the relationship between the second exposure and the illness; in this case, the relationship of the second exposure to the illness changes with the level of the first exposure.

The issue of interaction is relevant to the question of the combined effect of two or more exposures (Koopman, 1977; Kupper and Hogan, 1978; Rothman, 1974, 1978). Several kinds of statistical models are available for assessing interaction, but there is some dispute over which is the most appropriate (Kupper and Hogan, 1978; Rothman, 1974; Walter and Holford, 1978). Until more is known about how each exposure causes illness or produces biologic effects, the relevance of these statistical models to the biology of illness is moot.

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